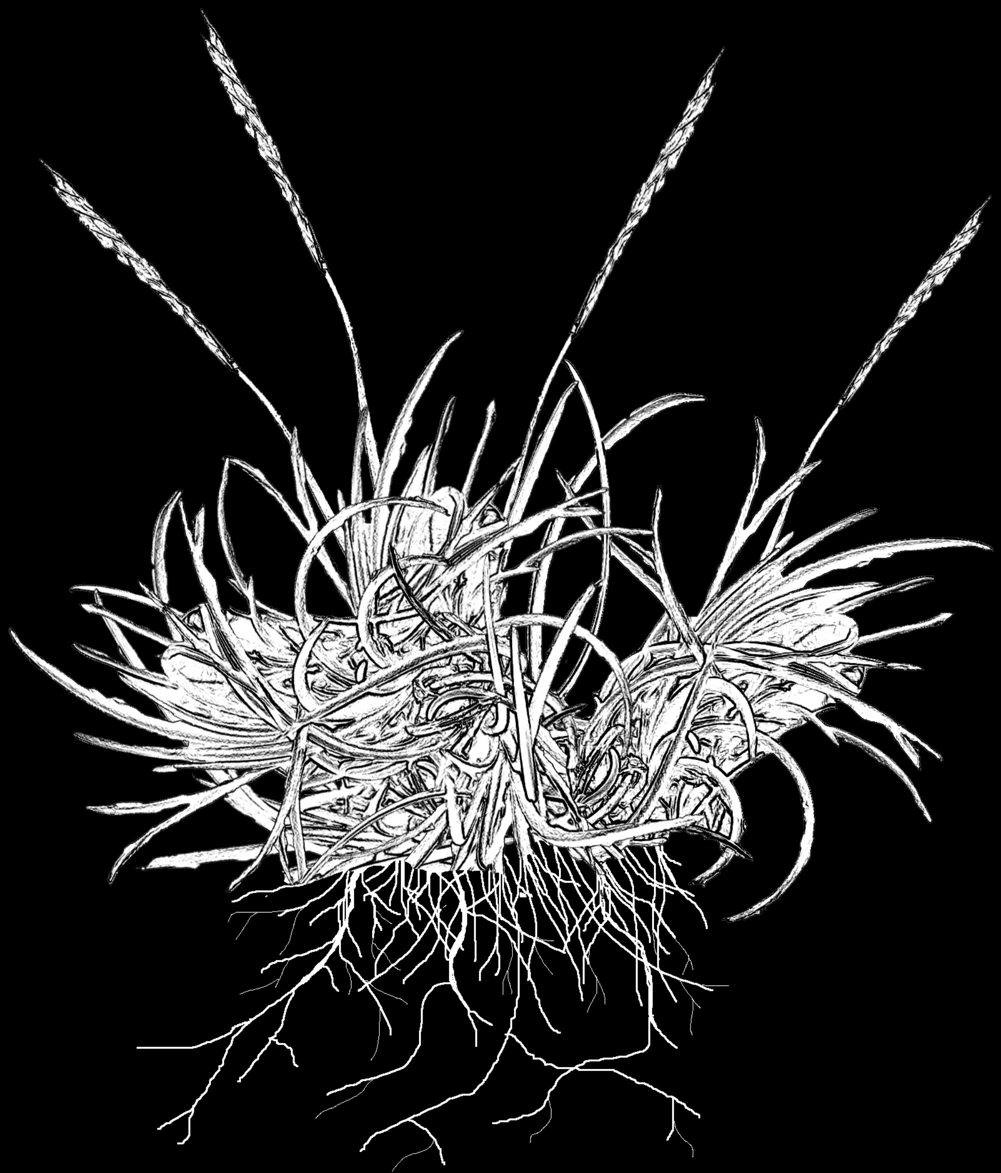

Patrones de ensamblaje en comunidades vegetales de alta montaña Mediterránea

Pescador, D.S.

Universidad
Rey Juan Carlos

Patrones de ensamblaje en comunidades vegetales de alta montaña Mediterránea

David Sánchez Pescador
Tesis Doctoral
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ÁREA DE
BIODIVERSIDAD
Y CONSERVACIÓN

Dr. Adrián Escudero Alcántara, Catedrático del Departamento de Biología y Geología de la Universidad Rey Juan Carlos, y Dr. Fernando Valladares Ros, Profesor de Investigación del Museo Nacional de Ciencias Naturales perteneciente al Consejo Superior de Investigaciones Científicas,

CERTIFICAN

Que los trabajo de investigación desarrollados en la memoria de tesis doctoral "**Patrones de ensamblaje en comunidades vegetales de alta montaña Mediterránea**", son aptos para ser presentados el Ldo. David Sánchez Pescador ante el tribunal que en su día se consigne, para aspirar al Grado de Doctor en el Programa de Conservación de Recursos Naturales por la Universidad Rey Juan Carlos de Madrid.

VºBº Director de Tesis	VºBº Director de Tesis
Dr. Adrián Escudero Alcántara	**Dr. Fernando Valladares Ros**

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TESIS DOCTORAL

Patrones de ensamblaje en comunidades vegetales de alta montaña Mediterránea

DAVID SÁNCHEZ PESCADOR

Directores:

Dr. Adrián Escudero Alcántara

Dr. Fernando Valladares Ros

Departamento de Biología y Geología

Área de Biodiversidad y Conservación

Universidad Rey Juan Carlos

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RESUMEN

INTRODUCCIÓN

El concepto de ecología de comunidades representa el estudio en conjunto de especies que coexisten en un espacio y tiempo determinado. Uno de los objetivos de la ecología de comunidades es la búsqueda de las reglas subyacentes que determinan esa coexistencia (MacArthur, 1972). Desde el nacimiento de la Ecología de Comunidades de plantas los intentos para explicar estos patrones han derivado en numerosas y contrapuestas hipótesis. El debate casi comienza en los albores de la Ecología con una histórica controversia de los botánicos Clements (1916) y Gleason (1926). El primero puso en valor el término ‘organismo complejo’ para reflejar como las comunidades de plantas están limitadas por una serie de procesos deterministas, existiendo sólo ciertas combinaciones de especies posibles. El segundo veía las interacciones entre especies como el resultado de procesos estocásticos o simplemente ‘coincidencia’, al considerar las comunidades como colecciones aleatorias de especies con facultades para persistir en un área y tiempo determinados. Ambas visiones representan en la actualidad los extremos de un gradiente conceptual donde se hallan las respuestas al ensamble de las comunidades (Lortie *et al.*, 2004; Kembel, 2009).

El avance en el campo de la Ecología de Comunidades ha sido notorio desde su aparición y aunque toda una discusión sobre los mecanismos que hay detrás del ensamble de comunidades ha sido mantenida desde los años 80 (Ricklefs, 1987; Cornell & Lawton, 1992; Keddy, 1992; Belyea & Lancaster, 1999; Hubbell, 2001; Ackerly, 2003; Grime, 2006; Brooker *et al.*, 2009; Ricklefs, 2009) muchas preguntas quedan aún por resolver. Los principales procesos que han ido delimitando el paradigma que concierne al ensamblaje de las comunidades son de diferente naturaleza y actúan a diferentes escalas espacio-temporales (Zobel, 1997). A una escala más regional los procesos históricos ligados a especialización, extinción y migración de las especies (Ricklefs, 2004) además de procesos de estocasticidad demográfica (Tilman, 2004) conforman el ensamble regional de especies. A partir de este ‘*pool’* regional las especies se van organizando en las comunidades de acuerdo a lo que se han venido a denominar las reglas ecológicas de ensamblaje. Este término fue acuñado en ecología por Diamond (1975) quién tras analizar las combinaciones de aves en diferentes celdas espaciales concluyó que la interacción entre las especies, especialmente la competencia, derivaba en patrones no aleatorios de coexistencia. Esta idea puso en juego un concepto más antiguo, la exclusión competitiva. Pese al carácter competitivo atribuido a la coexistencia de especies inicialmente otros autores han defendido la presencia de procesos selectivos que actúan como una serie de filtros o barreras ecológicas limitando de manera jerárquica las especies que pueden coexistir en las comunidades (Grime, 1979; Keddy, 1992). Una visión más neutral rebate cualquier diferencia ecológica entre las especies y limita el ensamble a meras cuestiones estocásticas o relacionadas sólo con procesos dispersivas (Hubbell, 2001). Así las especies pueden ser restringidas por una limitación en su dispersión por las limitaciones impuestas por el ambiente o por las interacciones bióticas, no sólo competitivas si no de cualquier otra naturaleza.

Pese a la simplicidad de este esquema actualmente continuamos encontrando ciertas discrepancias en la bibliografía a la hora de adaptar estas reglas a las comunidades naturales. Soluciones a un mismo problema se han descrito por ejemplo basándose en los rasgos funcionales de las especies que forman parte de una misma comunidad. Así pues éstas pueden denotar una convergencia en los rasgos funcionales si un filtro ambiental limita su supervivencia (Grime, 1979; Keddy, 1992). Por ejemplo el frío en las comunidades alpinas condiciona a que las especies que ellas habitan presenten una serie de serie de caracteres adaptativos ‘necesarios’ (Billings, 1974). Sin embargo una ‘*limiting similarity’* (*sensu* MacArthur & Levins, 1967) provocaría una divergencia en los rasgos funcionales para limitar la competencia entre especies de requerimientos similares (Mason & Wilson, 2006). Estas aparentes discrepancias se han extendido al ámbito evolutivo de las comunidades y así podemos hablar de una de ‘*overdispersion*’ o ‘*underdispersion*’ filogenética con una explicación análoga a la dada para los rasgos funcionales (Silvertown *et al.*, 2001; Webb *et al.*, 2002; Cavender-Bares *et al.*, 2004; Verdú & Pausas, 2007).

Dos poderosas aproximaciones se han desarrollado recientemente para evaluar los mecanismos subyacentes a la construcción y dinámica de las comunidades (Kraft *et al.*, 2007; Swenson & Enquist, 2009; Baraloto *et al.*, 2012; Purschke *et al.*, 2013; Swenson, 2013; Wang *et al.*, 2013). En concreto el estudio de los rasgos funcionales y de las relaciones evolutivas o filogenéticas ha sido propuesto como el camino a seguir para de inferir las respuestas al desafío de buscar los mecanismos de ensamblaje. Ambas aproximaciones se basan en comparar las similitudes o disimilitudes de las especies presentes dentro de una comunidad y entre comunidades y a diferentes escalas espacio-temporales. En el primer caso los rasgos funcionales representan cualquier característica morfológica, fisiológica o ecológica medible en un individuo y que directa o indirectamente repercute en su habilidad para sobrevivir en un ambiente determinado (Violle *et al.*, 2007). Estas propiedades por lo tanto se presentan como un reflejo de las estrategias ecológicas de las especies (McGill *et al.*, 2006), lo que ha llevado a asumir que un limitado número de rasgos funcionales bastan para describir la distribución de las especies a lo largo de los gradientes ambientales (Diaz *et al.*, 2004; Shipley *et al.*, 2006; Spasojevic *et al.*, 2014). Aproximaciones basadas en los rasgos funcionales han sido propuestas para demostrar la importancia del filtrado ambiental y de la '*limiting similarity'* en multitud de comunidades de plantas (Cornwell *et al.*, 2006; Kraft *et al.*, 2008; Cornwell & Ackerly, 2009; Spasojevic & Suding, 2012; de Bello *et al.*, 2013a). Pese a ello las principales conclusiones al respecto están sujetas a la importancia de la dependencia de los rasgos a los mecanismos subyacentes (Maire *et al.*, 2012), la variabilidad intra-específica (Albert *et al.*, 2010a; Albert *et al.*, 2010b; Auger & Shipley, 2012) y el efecto de '*turnover'* o reemplazo de especies en las comunidades (Cornwell & Ackerly, 2009; Lepš *et al.*, 2011). Todo esto puede implicar ciertas contradicciones a la hora de predecir patrones, lo que implica que los rasgos funcionales no se muestren como la panacea para explicar los mecanismos de ensamblaje. Esto llevó a Mayfield and Levine (2010) a cuestionar la asunción de que el mecanismo de competencia siempre elimina especies más parecidas funcionalmente y consecuentemente a re-interpretar los mecanismos de ensamble desde un punto de vista filogenético.

Los avances en la obtención de ADN y las mejoras en los métodos estadísticos (Webb & Donoghue, 2005; Pearse & Purvis, 2013) desarrollados desde la pasada década han permitido describir las relaciones evolutivas entre las especies de una comunidad y consecuentemente contrastar las teorías de coexistencia bajo esta perspectiva (Webb *et al.*, 2002). Como ya hemos indicado con anterioridad esta aproximación filogenética a la coexistencia que permite la consideración explícita de procesos evolutivos se basa en la idea de que especies emparentadas filogenéticamente tienden a ser ecológicamente similares (Losos, 2008), lo que ha llevado a la asunción de que las distancias filogenéticas representan un subrogado de las diferencias ecológicas de nicho (Cavender-Bares *et al.*, 2004; Kraft & Ackerly, 2010). No obstante esta aproximación tampoco está exenta de contracciones. Y así por ejemplo Cahill *et al.*

(2008) describió una limitada evidencia de competencia entre organismos emparentados filogenéticamente.

Todas estas contradicciones convierten la búsqueda de los patrones de ensamblaje en una misión ardua. La problemática posiblemente venga originada por una discrepancia en las escalas donde se ha focalizando la búsqueda (Kraft & Ackerly, 2010; Götzenberger *et al.*, 2012; Bernard-Verdier *et al.*, 2013; Chase, 2014). Así la solución a este fenómeno conocido como '*spatial grain size problem*' (Wiens, 1989; Levin, 1992) pasa por conocer cómo los mecanismos y dinámicas varían con la escala de descripción. Esto puede hacerse desde una un enfoque multiescalar, dónde los mecanismos se evalúan a lo largo de las diferentes escalas espaciales y/o temporales (Kraft & Ackerly, 2010; Messier *et al.*, 2010; de Bello *et al.*, 2013b) o a través del mapeo completo de toda la comunidad mediante técnicas estadísticas de análisis espacial. Recientemente estamos siendo testigos de una explosión de estudios que usan la estadística espacial en ecología para explicar los mecanismos subyacentes de ensamblaje (Wiegand & Moloney, 2004; Seabloom *et al.*, 2005; Perry *et al.*, 2006; Wiegand *et al.*, 2007; Law *et al.*, 2009; Wang *et al.*, 2011; Lan *et al.*, 2012). Estos métodos son especialmente útiles a la hora de revelar las escalas espaciales a las que ocurren los procesos ecológicos, tanto los ligados a una heterogeneidad ambiental (Getzin *et al.*, 2008; Shen *et al.*, 2013), así como los puramente relacionados con las interacciones bióticas y las limitaciones dispersivas (Amarasekare, 2003; Wiegand *et al.*, 2009). De esta manera una línea futura de trabajo debería de evaluar cómo la heterogeneidad ambiental, o más en concreto de la micro-heterogeneidad ambiental (Burton *et al.*, 2011), y las relaciones entre las especies, interacciones, se estructuran en el espacio y tiempo.

Los ecosistemas de alta montaña representan uno de 'laboratorio naturales' por excelencia donde testar los patrones de coexistencia de las especies. Estos ecosistemas se encuentran por encima del límite natural arbóreo, cubriendo aproximadamente el 5% de la superficie terrestre del planeta con una representación de alrededor 10.000 especies (Körner, 2003). La biota de estos ambientes se somete a abruptos gradientes ambientales a muy pequeñas escalas espaciales que determinan, en muchas ocasiones, cambios en la composición y estructura de las comunidades (Nagy & Grabherr, 2009). Así por ejemplo cambios altitudinales suponen para las plantas fuertes y predecibles variaciones térmicas, cambios de la época de crecimiento, radiación incidente y viento (Körner, 2007). Las bajas temperaturas representan uno de los principales filtros que las especies deben sobrepasar para sobrevivir en estos ambientes (Körner, 2003). Las plantas de hábitats alpinos cuentan así con toda una batería de adaptaciones fisiológicas y estrategias que favorecen la adaptación a estas condiciones (Billings, 1974; Bannister *et al.*, 2005; Beck *et al.*, 2007) y que pueden ser interpretadas como respuesta a dicho filtro. Otro patrón bien descrito en estos sistemas es el aumento de las interacciones positivas a medida que se asciende en altura (Kikvidze & Nakhutsrishvili, 1998; Callaway *et al.*, 2002), el cual ha sido descrito como uno de los principales conductores de la diversidad (Cavieres *et al.*, 2014). La severidad ambiental de estos sistemas determina que en muchos casos las plantas se organicen en estructuras caprichosas donde las plantas se agrupan de manera ordenada y no azarosa (Escudero *et al.*, 2004). Un ejemplo son los estructuras bifásicas donde las plantas confinadas en parches quedan embebidos en una matriz de suelo desnudo (Kikvidze, 1993; Nuñez *et al.*, 1999). Esta estructura es el resultado de la interacción real entre los individuos que conforman el parche. Dichas interacciones se pueden describir como redes de interacciones donde la búsqueda de patrones no azarosos como el anidamiento (Patterson & Atmar, 1986; Atmar & Patterson, 1993; Bascompte *et al.*, 2003) o la modularidad (Guimera & Amaral, 2005; Lewinsohn *et al.*, 2006; Olesen *et al.*, 2007) puede ayudar a conformar una nueva teoría de la coexistencia.

Un caso peculiar de este tipo de ambientes es el de la alta montaña Mediterránea. La peculiaridad de estos sistemas radica en el déficit hídrico al que se ven sometidas sus especies durante la corta estación en el cual las temperaturas son lo suficientemente altas como para permitir el crecimiento (Giménez-Benavides *et al.*, 2007). Esto puede representar un filtro adicional para estas comunidades motivando desajustes en el ensamble de especies e incluso en las interacciones (Cavieres *et al.*, 2006; Schöb *et al.*, 2013). La alta montaña mediterránea se presenta así como una excelente oportunidad en la que explorar la importancia de los procesos locales (interacciones y micro-heterogeneidad ambiental) y regionales (gradientes ambientales) en la configuración de las comunidades de plantas. Un valor añadido al estudio de estos sistemas reside en la vulnerabilidad que presentan los mismos ante el Cambio Global (Thuiller *et al.*, 2005; Gottfried *et al.*, 2012). Los modelos de cambio climático vigentes pronostican un incremento en las temperaturas y descenso en las precipitaciones más acentuado en las montañas de clima mediterráneo de la Península Ibérica (Nogués-Bravo *et al.*, 2008; Engler *et al.*, 2011). Además el ascenso de los cinturones arbustivos de altitudes menores debido al calentamiento global así como a la caída de la cabaña ganadera hace que las comunidades de alta montaña mediterránea vean reducida su área año a año (Sanz-Elorza *et al.*, 2003). Todo ello convierte a la alta montaña mediterránea en un potencial laboratorio donde testar los efectos de este Cambio Global.

OBJETIVOS

El objetivo principal de esta tesis es la evaluación de las reglas ecológicas de ensamblaje y los mecanismos que afectan a las comunidades de plantas perennes sometidas a una fuerte severidad ambiental como son las comunidades vegetales de alta montaña Mediterránea. Considerando las dificultades de escala planteadas en la literatura, a la hora de detectar estas reglas se tuvo en cuenta un enfoque multiescalar desde el punto de vista ambiental, funcional, filogenético y espacial. Para la consecución de este objetivo se desarrollaron los siguientes objetivos específicos:

- Dado que el frío debe ser el principal filtro para poder vivir en la alta montaña y especialmente la resistencia a éste durante la corta ventana en la cual hay actividad vegetativa un primer objetivo es estudiar el potencial papel de los eventos estivales de congelación, considerados como un filtro abiótico primario, en el ensamble de la comunidad vegetal de alta montaña Mediterránea, así como describir los principales mecanismos y estrategias implicados en la resistencia al frío.

- Evaluar cómo los rasgos funcionales de la comunidad vegetal de alta montaña responden a cambios en las condiciones ambientales y en concreto a la altitud, centrándose en la relevancia de los diferentes recursos de variabilidad funcional: ‘dentro de -’ frente a ‘entre-comunidades’ e ‘intra-’ frente a ‘inter-específico’.

- Establecer la importancia de la escala en el estudio de los mecanismos de ensamblaje de comunidades así como los patrones de convergencia y divergencia desde un punto de vista funcional y filogenético, evaluando la importancia de los recursos de variabilidad a diferentes escalas.

- Analizar la presencia de los patrones de anidamiento y modularidad en las estructuras bifásicas (mosaicos de parches de vegetación y suelo desnudo) características en la alta montaña Mediterránea, así como evaluar su respuesta ante los principales gradientes ambientales y estructurales.

- Estimar los efectos bióticos que las especies dominantes en las comunidades vegetales de alta montaña ejercen sobre la distribución y abundancia del resto de especies desde una perspectiva espacial.

MATERIAL Y MÉTODOS

En cada uno de los capítulos que prosiguen a esta memoria se desarrollan en profundidad la metodología, material y análisis estadísticos empleados para la consecución de los objetivos planteados. No obstante en los siguientes apartados se describirá de manera esquemática el área y sistema de estudio, el diseño de muestreo y los principales análisis estadísticos llevados a cabo.

Área de estudio

Todos los capítulos que conforman esta tesis doctoral se han centrado en las comunidades psicroxerófilas de alta montaña mediterránea (Fig. 1) localizadas por encima del límite arbóreo en la Sierra de Guadarrama, una cadena montañosa con cerca de 100 km perteneciente al Sistema Central y situada a 70 km al noroeste de Madrid, España (Fig. 3; coordenadas en el extremo suroeste 40º46´39´´, 4º4´59´´O y extremo noreste 40º51´8´´ N, 3º49´44´´). Concretamente la mayoría de las ubicaciones seleccionadas para llevar a cabo el trabajo de campo estuvieron englobadas dentro del Parque Nacional de Sierra de Guadarrama y del Parque Regional de la Cuenca Alta del Manzanares.

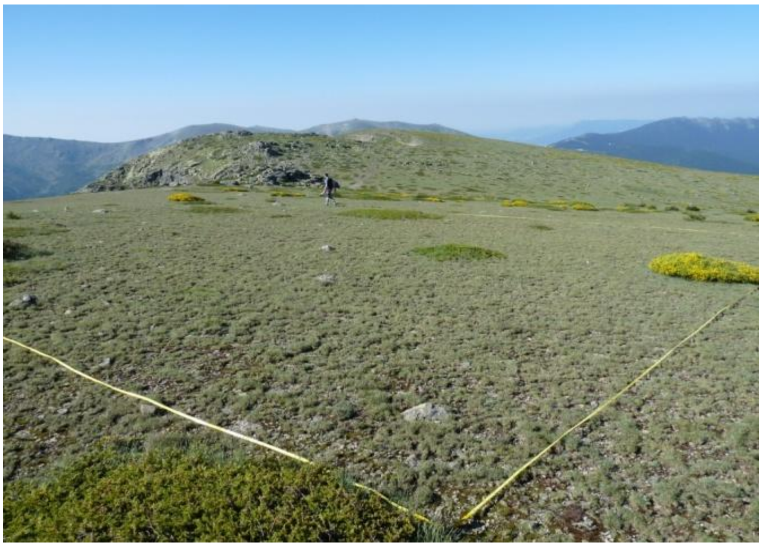


Figura 1. Comunidades psicroxerófilas de alta montaña Mediterránea dominadas por *Festuca curvifolia* con algunos arbustos de *Cytisus oromediterraneus* y *Juniperus communis* subsp. *alpina* en las inmediaciones de Peñalara, Prov. de Madrid.

El clima en esta región montañosa es Mediterráneo caracterizado por una temperatura y precipitación media anual de 6,4 ºC y 1318 mm respectivamente (Tabla 1). Una peculiaridad de los sistemas montañosos Mediterráneos es la marcada sequía registrada de junio a octubre que representa menos del 10% de la precipitación anual y es más patente a bajas altitudes (Palacios *et al.*, 2003; Giménez-Benavides *et al.*, 2007).

Tabla 1. Información climática referente al área de estudio comprendida para la serie temporal de 1946- 2011 procedente de la estación meteorológica del Puerto de Navacerrada (40°47' 35" N, 4°0' 38" W; 1896 m). Recurso: *Agencia Estatal de Meteorología - Ministerio de Agricultura, Alimentación y Medio Ambiente* de España.

	Anual	Estación de crecimiento	Mayo	Junio	Julio	Agosto	Sept.
Temperatura media del aire (°C)	6.48	12.88	7.05	12.29	16.42	16.20	12.43
Temperatura media mínima del aire (°C)	2.77	8.18	3.09	7.65	11.15	10.96	8.06
Temperatura mínima del aire absoluta (ºC)	-20.30	-8.00	-8.00	-3.40	0.00	0.20	-3.00
Temperatura media máxima del aire (°C)	10.18	17.56	11.00	16.90	21.67	21.42	16.79
Temperatura máxima del aire absoluta (ºC)	31.80	31.80	25.40	29.00	30.80	31.80	30.80
Precipitación media (mm/mes)	109.86	64.61	128.52	69.11	24.19	27.55	71.33
Promedio de días de lluvia	67.45	33.17	8.34	8.24	4.42	5.20	8.40
Promedio de días de nieve	72	6	5	1	0	0	0
Promedio de días de granizo	5	7	3	2	1	1	1
Insolación media (horas/día)	6.07	8.99	6.97	9.21	11.16	10.40	7.13
Insolación (%)	48.09	63.52	48.02	61.26	75.58	75.41	57.00
Días/mes con Tª < 0ºC	12	2	8	1	0	0	1

La litología granítica y gnéisica domina la Sierra de Guadarrama lo que condiciona una flora de carácter silicícola con pocas variaciones edafológicas. No obstante limitados afloramientos calcáreos a lo largo de la zona septentrional y meridional de la sierra enriquecen la flora con elementos de carácter calcícola (aunque las comunidades estudiadas no se centraron en éstas). Los suelos del área de estudio están dominados por leptosoles (úmbricos y lítico) y cambisoles húmico con suelos franco arenosos de pH acido.

El límite altitudinal del dosel arbóreo en la Sierra de Guadarrama se extiende hasta los 1900-2000 m de altitud y está representado básicamente por el pino silvestre (*Pinus* *sylvestris* L.). Por encima de este límite el cinturón oro-mediterráneo (2100 – 2200 m) está dominado por una matriz arbustiva de piorno serrano (*Cytisus oromediterraneus* Rivas Mart. *et al*.) y enebro rastrero (*Juniperus communis* L. subsp. *alpina* (Suter) Čelak) con presencia de pastos psicroxerófilos en las localidades menos pronunciadas. En las áreas más elevadas del nuestra área de estudio (cinturón criomediterráneo, desde los 2200 – 2428) se extienden las comunidades de pastos psicroxerófilos especialmente en las cumbres y zonas venteadas libres de neveros.

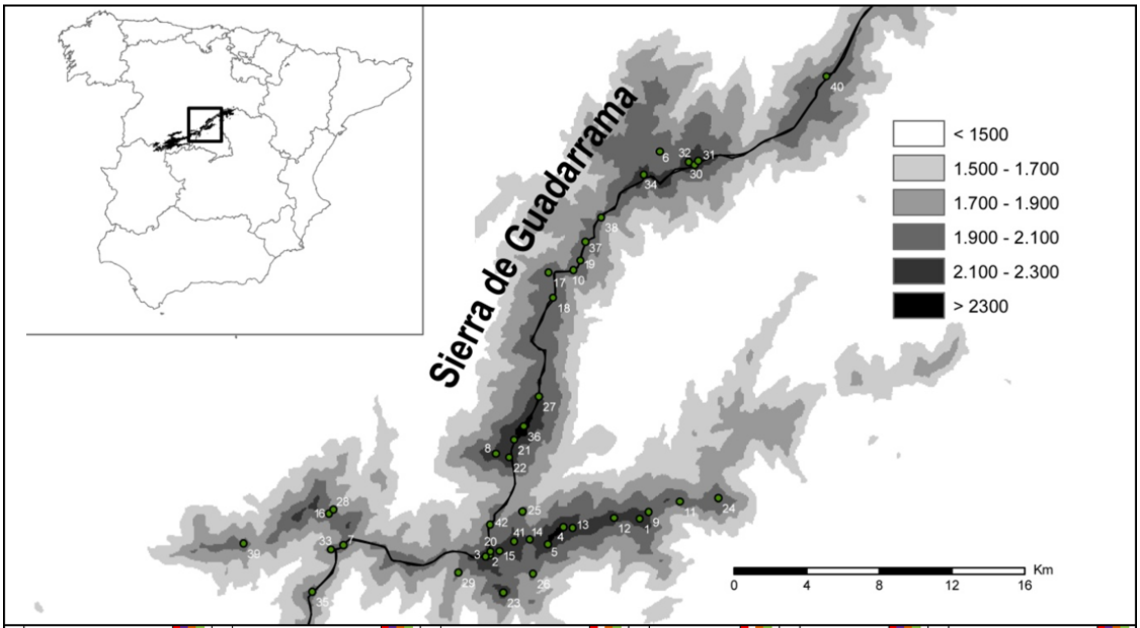
Fig. 2. Graderío característico de las comunidades psicroxerófilas de alta montaña Mediterránea en las inmediaciones de Bola del Mundo, Prov. de Madrid.

La comunidad de pastos psicroxerófilos está caracterizada por las condiciones que definen estos ambientes, el frio y la sequedad durante el verano, lo que la convierte en una comunidad singular de especies perennes de bajo porte dominadas por hemicriptófitos graminoides y caméfitos postrados y almohadillados. Los fenómenos de crioturbación y solifluxión durante el periodo de actividad vegetativa son acusados lo que a menudo generan una micro-topografía de graderío con bandas (Fig. 2). La cobertura de pasto es muy variable y dependiente de la inclinación y grado de matorralización de las localidades. La dominancia de la gramínea *Festuca curvifolia* Lag. *ex* Lange es patente en la mayoría de los enclaves donde es acompañada por todo un elenco florístico rico en endemismos locales tal como *Jasione crispa* (Pourr.) Samp.

subsp. *centralis* (Rivas Mart.), *Pilosella vahlii* (Froel.) F.W. Schultz & Sch. Bip., *Minuartia recurva* (All.) Schinz & Thell., *Armeria caespitosa* (Gómez Ortega) Boiss. in DC. o *Erysimum penyalarense* (Pau) Polatschek. junto con algunos elementos ártico-alpinos, como *Agrostis rupestris* (All.) y *Phyteuma hemisphaericum* L., que alcanzan aquí su límite latitudinal sur.

Diseño de muestreo

El protocolo de trabajo que se ha desarrollado en los diferentes capítulos es específico y su descripción queda detallada en cada uno de los mismos. No obstante manera general a lo largo del gradiente altitudinal de la Sierra de Guadarrama se estableció en el verano de 2011 una red de de 42 localidades (ver Fig. 3) dominadas por la comunidad psicroxerófila de alta montaña Mediterránea. La selección de las diferentes localidades se hizo en base a las ortofotos del Plan Nacional de Ortofotografía Aérea (PNOA; http://www.ign.es/PNOA/) y en el marco de un Sistema de Información Geográfica (ArcGis 10.2 software – ESRI, 2011) evitando zonas cóncavas con compensación edáfica o zonas de ventisquero donde se acumula la nieve y donde se producen cambios muy llamativos en la composición y dominancia de las especies. En cada caso la elección de los lugares se realizó cubriendo no sólo el rango de altitud completo sino aquellos componentes más conspicuos de heterogeneidad ambiental, como puede ser la orientación, el nivel de matorralización o la pendiente. En la figura 4 podemos ver un esquema del muestreo que se llevó a cabo para obtener datos de composición y estructura de estas comunidades a diferentes escalas espaciales.



1	Asomate-Hoyos	🟥🟪🟧🟩
2	Bola del Mundo	🟥🟧🟩
3	Bola del Mundo Oeste	🟥🟪🟧🟩🟦
4	Cabeza de Hierro Mayor	🟥🟪🟧🟩
5	Cabeza de Hierro Menor	🟥🟧🟩
6	Cerro Malo	🟥🟩
7	Cerro Minguete	🟥🟧🟩
8	Citores	🟥🟪🟧🟩
9	Collado de Bailanderos	🟥🟧🟩
10	Collado de la Flecha	
11	Collado de la Najarra	🟥🟧🟩
12	Collado de las Zorras	🟥🟧🟩
13	Collado de Peña Vaqueros	🟥🟧🟩
14	Collado de Valdemartin	🟥🟧🟩
15	Collado de las Guarramillas	🟥🟧🟩
16	Collado Tirobarra	🟥🟪🟧🟩
17	El cancho	
18	El Reventón	🟥🟩
19	Flecha	🟥🟧🟩
20	Guarramillas	🟥🟧🟩
21	Hermana Mayor	🟥🟪🟧🟩
22	Hermana Menor	🟥🟧🟩
23	La Maliciosa	🟥🟧🟩
24	La Najarra	🟥🟪🟧🟩
25	Las Cerradillas	🟥🟧🟩
26	Loma Cabezas	
27	Los Claveles	🟥🟧🟩
28	Los Horcajos	🟥🟧🟩
29	Navacerrada	🟥🟪🟧🟩
30	Nevero	🟥🟧🟩
31	Nevero Este	🟥🟧🟩
32	Nevero Oeste	🟥🟧🟩
33	Peña Bercial	🟥🟧🟩
34	Peña Cabra	🟥🟧🟩
35	Peña del Aguila	🟥🟧🟩
36	Peñalara Norte	🟥🟪🟧🟩
37	Puerto de las Canderuelas	🟥🟧🟩
38	Puerto de Malagosto	🟥🟧🟩
39	Puerto del Pasapan	🟥🟧🟩
40	Reajo Alto	🟥🟧🟩
41	Valdemartin	🟥🟧🟩
42	Loma Noruego	🟥🟧🟩

Fig. 3. Mapa de la Sierra de Guadarrama en el Sistema Central donde se representa la ubicación de las parcelas establecidas en las comunidades psicroxerófilas de alta montaña dominadas por *Festuca curvifolia*. En código de colores delimita las parcelas empleadas en el Capítulo 1 (rojo), Capítulo 2 (morado), Capitulo 3 (naranja), Capítulo 4 (verde) y Capítulo 5 (azul).

En cada localidad se instaló una parcela, de 20 m de lado, y se realizó un primer inventario de todas las especies presentes estimando su cobertura aérea de manera visual (**Capítulos 1 y 3**). Para analizar la estructura que presenta la comunidad (**Capítulo 4**) en cada parcela se localizaron 4 transectos lineales de 6,25 m siempre siguiendo la orientación de la pendiente, sumando un total de 25 metros lineales por parcela. En cada transecto se registró la intercepción, identidad y dimensiones de cada parche de vegetación o suelo desnudo así como las especies acompañantes en cada caso. A su vez se analizaron los diferentes patrones de coexistencia inter-especifica (**Capítulo 3**) gracias al monitoreo de la cobertura de cada especie presente en cada una de las 64 celdillas de 30 cm de lado que formaban un cuadrado de 2,4 m de lado. En este cuadrado y en otros cuatro adicionales de semejantes dimensiones y aleatoriamente emplazados se registró la cobertura total cada una de las especies vegetales presentes así como de roca y suelo desnudo. Finalmente, en cada una de las parcela se recogieron de manera estratificada 15 muestras de suelo (5 cm de diámetro por 10 cm de profundidad) en tres microambientes: zonas desnudas (B en Fig. 4), zonas de pastos de gramíneas (P en figura 2) y, zonas bajo la influencia de los arbustos *Cytisus oromediterraneus* y *Juniperus communis* subsp. *alpina* (A en Fig. 4). Los suelos recogidos fueron empleados para la caracterización edáfica de cada localidad y sus rangos empleados en todos los capítulos de la memoria.

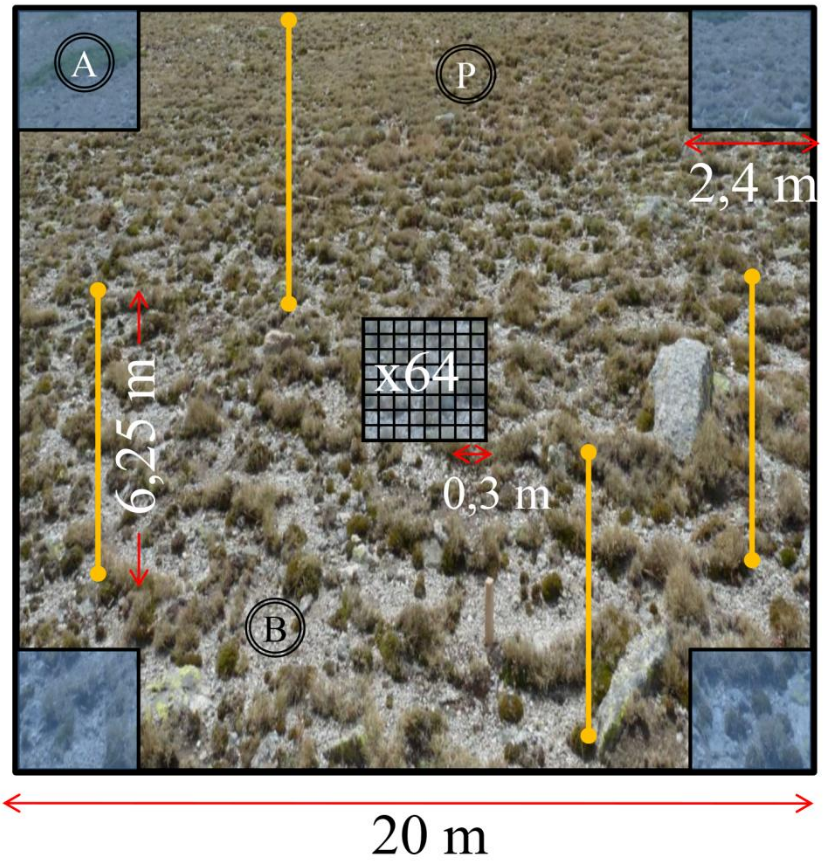


Fig. 4. Esquema de muestreo para una parcela básica de la red de localidades muestreadas en la Sierra de Guadarrama. Las cuatro escalas de trabajo son incluidas: a) cuatro transectos para la estimación de la estructura de la comunidad (**Capitulo 4**); b) parcela de 400 m^2; c) cinco parcelas de 2.4 x 2.4 m; d) parcela central donde se estableció una malla rectangular de 64 celdas de 0,3 x 0,3 m. Las letras P de "Pasto", B de "Bare" y A de "Arbusto" reflejan cada uno de los micro-ambientes donde se recogieron las muestras de suelo.

Análisis estadísticos

En los diferentes capítulos las técnicas estadísticas empleadas fueron de una amplia índole en respuesta a las diferentes preguntas planteadas. La gran mayoría de las variables empleadas en los diferentes capítulos fueron continuas por lo que los modelos lineales simples y generalizados (GLMs) fueron empleados con carácter dominante (**Capítulos 1, 2, 3 y 4**) con el objetivo de explicar la relación directa entre diferentes variables continuas dependientes y sus variables explicativas. Para el análisis de las relaciones indirectas entre las diferentes variables se emplearon modelos de ecuaciones estructurales (SEM; **Capítulo 4**). Análisis multivariantes fueron usados en el **Capítulo 2** para estudiar simultáneamente múltiples características en cada uno de los individuos objetos de investigación. En el **Capítulo 3** se recurrió a estadística bayesiana para resolver las relaciones filogenéticas entre las especies que conforman la comunidad de pastos psicroxerófilos de Guadarrama. Finalmente en el **Capítulo 5** se recurrió a una estadística espacial compleja usando diferentes herramientas tales como los análisis del vecino más próximo para evaluar los patrones espaciales que conforman las comunidades.

La mayoría de los análisis estadísticos realizados durante el desarrollo de esta tesis se han realizado bajo el lenguaje de programación R (R Core Team, 2012) y recurriendo al uso de alguna de sus múltiples librerías (ade4, bipartite, ecespa, FD, lme4, MuMIn, nlme, picante, spatstat o vegan entre otras).

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AFILIACIÓN DE LOS COAUTORES (por orden alfabético)

de Bello, Francesco: Institute of Botany, Czech Academy of Sciences, CZ-379 82, Trebon, Czech Republic - Department of Botany, University of South Bohemia, Na Zlaté stoce 1, 370 05 České Budějovice, Czech Republic.

Chacón, Julia: Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, ES-28933 Móstoles, Spain.

De la Cruz, Marcelino: Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, ES-28933 Móstoles, Spain.

Escudero, Adrián: Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, ES-28933 Móstoles, Spain.

Iriondo, Jose María: Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, ES-28933 Móstoles, Spain.

Sierra-Almeida, Ángela: ECOBIOSIS, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Casilla 160-C, Concepción, Chile - Instituto de Ecología y Biodiversidad (IEB), Casilla 653, Santiago, Chile.

Torres, Pablo: Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, c/ Tulipán s/n, ES-28933 Móstoles, Spain.

Valladares, Fernando: Museo Nacional de Ciencias Naturales, MNCN, CSIC. Serrano 115 Bis, ES-28006 Madrid, Spain.

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- **CAPÍTULO II: Plant trait variation along an altitudinal gradient in Mediterranean high mountain grasslands: controlling the species turnover effect**

  Pescador, D.S., de Bello, F., Valladares, F. and Escudero, A.

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- **CAPÍTULO III: Local heterogeneity modulates environmental filtering in a Mediterranean high mountain community**

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- **CAPÍTULO IV: Plant to plant networks in a Mediterranean high mountain community: dominance of Nestedness over Modularity**

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- **CAPÍTULO V: Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy**

  Pescador, D., Chacón, J., de la Cruz, M. & Escudero, A.

  Manuscrito aceptado en New Phytologist

I

Summer freezing resistance, a critical filter for the plant community assembly of Mediterranean high mountains

Pescador, D.S., Sierra-Almeida, A., Torres, P.J., Escudero, A.

Manuscrito enviado a Oecologia

ABSTRACT

- *Background and Aims* Extreme low temperatures events during growth season may be an abiotic filter of alpine communities, which can interact with summer drought in Mediterranean mountains. How freezing resistance strategies affect the whole community assemblage in these Mediterranean stressful habitats remain unexplored.

- *Methods* We measured the leaves freezing resistance of 42 plants from Sierra de Guadarrama (Spain), by measuring their ice nucleation temperature (NT), freezing point (FP), low temperature damage (LT_{50}) and assigning the mechanism of freezing resistance (avoidance, tolerance and sensitive). We established the relation of these traits with four additional and key plant functional traits (i.e. plant height, specific leaf area, leaf dry matter content and seed mass) and growth forms. The freezing response of the community was estimated as community weighted means and functional diversity in 39 plots and the effect of altitude, insolation coefficient, shrub and pasture covers evaluated.

- *Key Results* We found a wide range of freezing resistance responses, from -18.6 to -5.1ºC. Almost a half of species was classified as freezing tolerant (47.6%) while a 42.9% as freezing avoidant, with freezing tolerant proportion increased along altitude. Relationships between freezing resistance traits and other functional traits were weak and only leaf dry matter content was negatively correlated with freezing resistance traits. At the community level, community weighted means of FP and LT_{50} showed a negative response with altitude and positive with shrub and pasture covers, while the functional diversity of both freezing traits increased along the altitude.

- *Conclusions* The results suggest that abiotic filtering by summer freezing events is relevant in the assembly of Mediterranean high mountains, where a concomitant summer drought could explain the high freezing resistance of species that thrive in there. This adaptation to drought seems to be a general condition for plant occurring in xeric mountains.

Key words: abiotic filters, alpine plants, community assembly, community weighted means, drought, freezing resistance, functional diversity, Mediterranean high mountains.

INTRODUCTION

Resistance to summer freezing temperatures has been suggested as the primary abiotic filter to inhabit in high elevation habitats (Körner, 2003) since extreme low temperature episodes during the growing season are the norm up there (Billings, 1974). This physiological trait is dependent of ambient conditions in which plants thrive (Bannister *et al.*, 2005; Beck *et al.*, 2007), and confers to some alpine plants the ability to resist temperatures near -20°C (Squeo *et al.*, 1996; Sierra-Almeida *et al.*, 2009; Venn *et al.*, 2013). Freezing temperatures during the winter are not critical simply because cold hardening and mainly snow cover provide protection to most high mountain plants (Inouye & McGuire, 1991; Bannister, 2005; Bannister *et al.*, 2005). However frost damage during the growing season is critical for survival and growing in these stressful habitats (Körner, 2003; Taschler & Neuner, 2004). An operative plant classification in terms of freezing plant resistance groups plants in two rough categories: plants with freezing avoidance (FA) and with freezing tolerance mechanisms (FT). In the first case, plant responses are based on prevention of ice formation through supercooling or freezing point depression (Burke *et al.*, 1976; Guy, 2003), and they are common among plants occurring where freezing events are moderate and of short duration (Sakai & Larcher, 1987). In the second, plants can survive extracellular freezing and frequently grow where freezing events are severe and of long duration (Sakai & Larcher, 1987). As a general pattern freezing tolerant species become dominant with altitude (Squeo *et al.*, 1996; Sierra-Almeida *et al.*, 2009). In addition, freezing-risk and mechanism relationship is based on a the existence of a thermal gradient in the air–soil profile, where colder air masses are accumulated near soil surfaces, exposing plant species growing near the soil surface to lower and longer freezing temperatures (Larcher, 2003).

For example, tropical and subtropical high mountain plant species with tall growth forms including giant rosettes (Rada *et al.*, 1985; Squeo *et al.*, 1991; Squeo *et al.*, 1996) are FA and resist higher freezing temperatures than small plants (<20 cm), such as some perennial herbs, rosettes and cushion plants, which are FT and resist lower freezing temperatures (Azócar *et al.*, 1988; Squeo *et al.*, 1991; Squeo *et al.*, 1996). Interestingly, this pattern does not occur among plant species from high mountains with Mediterranean climate in spite of the fact that a thermal gradient in the air-soil profile also exists (Sierra-Almeida *et al.*, 2010). Therefore, freezing resistance strategies among plant species with different growth forms is still an open question.

Most studies on freezing resistance in high mountain habitats have been conducted on temperate and high latitude mountains (Sakai & Otsuka, 1970; Neuner *et al.*, 1999; Taschler & Neuner, 2004; Körner & Alsos, 2008). However, plant life on Mediterranean mountains where the short growing season must be profoundly shaped by the existence of an additional stress due to water summer shortage (see Giménez-Benavides *et al.*, 2007) has not deserved studies on this topic (but see Loik and Redar, 2003; Sierra-Almeida *et al.*, 2009; Sierra-Almeida *et al.*, 2010). This is especially remarkable because freezing and drought responses are physiologically close (Beck *et al.*, 2007). For instance, osmotic adjustment by different metabolic procedures is usually involved in the response to both stresses (Anisko & Lindstrom, 1996; Blödner *et al.*, 2005; Nakashima & Yamaguchi-Shinozaki, 2006; Medeiros & Pockman, 2011). This suggests that the altitudinal response in these mountains could produce synergetic effects involving not only freezing but also drought responses. This co-occurrence probably would pose these plants among the most summer freezing resistant on the planet and could determine that known freezing strategies patterns in other mountains would not occur in Mediterranean mountains. In this sense, Mediterranean mountain specialists in Chile can resist temperatures below -19.5ºC during the growing season being the freezing tolerance the most frequent mechanism to withstand these events (Sierra-Almeida *et al.*, 2009). In addition, the relation between this physiological trait and other functional traits is not well known but a general pattern of an inverse relation between resistance and growth intensity (e.g. fast growing bulbs *vs.* slow growing cushion plants) seems to exist (Sakai & Larcher, 1987), but see (Sierra-Almeida *et al.*, 2010).

Despite abiotic filtering is thought to be the main coexistence mechanism in plant communities under very stressful conditions, to our knowledge the potential role of summer freezing resistance in the assembly of communities remains unexplored. Environmental or habitat filtering implies a certain level of trait convergence simply because abiotic conditions constrain those critical traits to certain values (Pavoine *et al.*, 2011). Our working hypothesis is that freezing events during growing season filter high mountain communities especially in Mediterranean-type climates where freezing risk runs parallel to the existence of summer drought. If true, the functional diversity in these traits must be reduced as altitude increase whereas the community weighted means (i.e. CWMs; Violle *et al.*, 2007; Lepš *et al.*, 2011) would drop with altitude simply because the probability of freezing temperatures increases with altitude (Nagy and Grabherr, 2009 p.23). These predictions can fail simply because there is an opposite gradient of summer drought with the highest intensity at low altitudes and also because the response to other local environmental factor such as exposure/insolation or structural factors such as the shrub cover (i.e. encroachment) can affect the final outcome (de Bello *et al.*, 2013).

With this in mind we have studied the summer freezing resistance of a Mediterranean high mountain community, determined the freezing resistance mechanisms (i.e. avoidance, tolerance or sensitive) and the potential role of this resistance in the assembly of these communities. Specifically, we tested whether: i) the proportion of freezing resistance mechanisms changes along altitude; ii) freezing resistance differences among growth forms occur in our system where a strong water summer shortage exists; iii) freezing strategies are correlated with other plant functional traits; iv) summer freezing events represent a abiotic filter affecting the response of community assembly.

MATERIALS AND METHODS

Study site and target species

The study was carried in cryophilic grassland communities in the Guadarrama National Park, approximately 70 km north-west of Madrid, Spain. The climate in this region is Mediterranean with 6.4ºC of average annual temperature and an average annual precipitation of 1318 mm in the Navacerrada Pass weather station (Supplementary Data Table S1; 40°46' N, 4°19' W; 1860 m a.s.l.), with an extreme summer drought (10% of the annual rainfall) which is more pronounced at lower altitudes (Giménez-Benavides *et al.*, 2007).

In summer of 2011 we sampled 39 cryophilic grasslands dominated by *Festuca curvifolia* Lag. ex Large. These communities are in the highest portions of Guadarrama National Park, above a timberline which is formed by stunted Scots pines (*Pinus sylvestris* L.) between 1900 and 2000 m interspersed in a shrubby matrix formed by *Cytisus oromediterraneus* Rivas Mart. & al. and *Juniperus communis* L. subsp. *alpina* (Suter) Celak. The sampled grasslands were distributed along the whole mountain range and covering the whole altitudinal gradient (1940 – 2428 m a.s.l.), different orientations, slopes and pasture and shrub covers. These communities are dominated by several creeping chamaephytes and caespitose grasses structured in a biphasic structure of plants conforming conspicuous patches or stripes into a bare ground matrix. In each grassland we established a plot of 20x20 m where cover of each plant species (discern between shrub cover and pasture cover) and percentage of bare soil were measured. We estimated the altitude and orientation using a GPS (Garmin Colorado-300) and the slope with a clinometer (Silva Clinomaster CM-360%, LA). Orientation and slope values allowed us to estimate the insolation coefficient by the Gandullo´s method (Gandullo, 1974; Pérez, 1997).

In 2012 we collected plant samples in four of the most diverse previous localities. The localities expand from the highest summit, Peñalara at 2419 m a.s.l to the Navacerrada Pass at 1860 m, and two else sites in the Bola del Mundo summit at 2242 m and Hermana Mayor Peak at 2280 m. A total of 42 plant species belonging to 17 families were sampled (Table 1). These species represent the most abundant species in the surveyed cryophilic grasslands and a wide phylogenetic and functional range. In particular, we sampled around of 65% of species present in this community, which cover over 90% of relative abundance. Plant species corresponded to four different growing forms: cushion chamaephytes (8), hemicryptophyte caespitoses (8), hemicryptophyte forbs (23) and erect shrubs (3).

Plant material was collected between 11 and 27 July 2012, during the peak of the growing season. Freezing resistance determinations were made in leaves randomly collected in the field from five mature individuals per species. Plant samples corresponded to small twigs with mature leaves for woody species (e.g. erect and cushion shrubs) and modules with at least two adult leaves or complete individuals for herbaceous species (e.g. hemicryptophyte caespitoses and forbs). Immediately after collection, plant samples were covered by wet paper and shipped individually in a tagged plastic bag, and kept into a cooler to avoid changes in tissue water status. Then, they were transported to the laboratory at the Rey Juan Carlos University. They were kept cold in a domestic refrigerator (i.e. 4°C) until freezing resistance determinations.

Freezing resistance determinations

Six expanded leaves from each plant sample were removed for freezing resistance determinations. We selected green fully expanded leaves to ensure that freezing resistance determinations were done in mature and active leaves. Leaves were used for both, thermal analyses and freezing temperature damage measurements as follow.

Thermal analyses

One leaf from each individual (five leaves by species) was taken to be analyzed in order to get the ice Nucleation Temperature (NT) and the Freezing Point (FP). Each leaf was bound to a thermocouple (Gauge 30 copper-constantan thermocouples; Cole Palmer Instruments, Vernon Hills, IL, USA) with parafilm and placed into a closed cryotube. The cryotubes were inserted into a cryostat (F34-ME, Julabo Labortechnik GmbH, Seelbach, Germany) that was programmed to decrease the temperature of the antifreeze solution (Husky, Würth, Germany) from 0ºC to -19ºC, at a cooling rate of 2ºC·h^{-1}. Temperature of individual leaves was live recorded every second with a Personal Daq/56 multi-channel thermocouple USB data acquisition module (IOtech, Cleveland, OH, USA) equipped with an expansion module PDQ2 and connected to a laptop. The sudden rise in leaf temperature (exotherm) produced by the heat released during the extracellular freezing process was used to determine the NT and the FP. The first temperature corresponds to the lowest temperature before the exotherm, indicating the onset of ice crystal formation. While the FP represents the highest point of the exotherm, indicating the freezing of water in the apoplast, including symplastic water driven outwards by the water potential difference caused by the apoplastic ice formation (Larcher, 2003).

Freezing temperature damage

For each species, five detached leaves from different plant samples were used in every round to estimate the freezing temperature damage (LT_{50}). Each leaf was placed into a marked small resalable plastic bag. Every single leaf bag belonging to the same species was placed into a bigger plastic bag. This procedure was used to separate and identify the different individuals of each species. It was necessary to take out the air from all the bags and to put some ballast to avoid flotation. Leaves were incubated in a previously cooled cryostat (F34-ME, Julabo Labortechnik GmbH, Germany). The cryostat was separately set in at four different temperatures: -5, -10, -15 and -19ºC. All leaves per round were transferred from the refrigerator to the cryostat and incubated during 2 h to reach homogeneous leaf temperatures. After that freezing treatment plastic bags were removed from the cryostat and placed back into refrigerator, under darkness and at 4ºC during 24 h for thawing. As control it was used leaves kept in darkness and stored in plastic bags at 4ºC during 24 h without the freezing treatment. This method of direct rather than gradual cooling and thawing (i.e. from 4°C to target temperatures and vice versa) thereby measures the current freezing resistance rather than the hardening capacity of plant material (Larcher *et al.*, 2010), and could have similar effects on plant leaves as several freezing events observed in the field. As visual damage was not immediately obvious for all species, leaf damage was assessed after thawing using a chlorophyll fluorimeter (Fluorescence Monitoring System FMS 2, Hansatech, Germany) to obtain the ratio of variable of maximum fluorescence (F_v/F_m) of dark-adapted leaf (Maxwell & Johnson, 2000). As dead leaf effectively had a F_v/F_m of zero (Bannister *et al.*, 2005), plant damage was calculated as percentage of photoinactivation (100 x *PhI*). Photoinactivation ratio was described by Larcher (2000) as *PhI* = (1-

F_{fT}/F_{max}) where F_{fT} is the F_v/F_m obtained for the sample exposed to freezing temperature T and F_{max} is the maximum F_v/F_m obtained of the individuals including the control. LT_{50} corresponds to the temperature in which *PhI* reaches a 50% value in leaf samples, and was determined by linear interpolation using the temperature of the highest *PhI* of <50% and the temperature of the lowest *PhI* of >50% (Bannister *et al.*, 1995; Bannister *et al.*, 2005). This method has been used in non-invasive surveys of thermal stress to photosynthesis, and it has been found to match results with other methods to assess LT_{50} (e.g. visual score and vital stain) in alpine (Neuner & Buchner, 1999), but also in chaparral plants (Boorse *et al.*, 1998).

We further compared our results with LT_{50} data of three available alpine plant studies. Specify, we selected those species corresponding to the four growth form in Austrian Alps (27 species ranged from 1950-2660 m; Taschler and Neuner, 2004), Chilean Andes (35 species between 2400 and 2900 m; Sierra-Almeida *et al.*, 2010) and Australian Alps (18 species at 1860 m; Venn *et al.*, 2013).

Freezing resistance mechanism

NT and LT_{50} for each species were compared to estimate the physiological freezing resistance mechanism. When LT_{50} was significantly lower than NT, the species is considered freezing tolerant (FT). In the other case, when LT_{50} was not significantly different from NT, species is considered freezing avoidant (FA) (Sakai & Larcher, 1987). In some plant species, LT_{50} occurred before NT and they were classified as freezing sensitive (FS).

Other Plant Functional Traits

The correlation among freezing resistance traits and other key functional traits was also evaluated. Thus, we measured four additional functional traits (see below) for most species sampled in thermal analyses (38 species; see Supplementary Data Table S2 for more details). Specifically we measured the plant height (Hmax, distance between the ground and the top photosynthetic tissues), specific leaf area (SLA; ratio between leaf fresh area and its dry leaf mass), leaf dry matter content (LDMC; ratio between leaf dry mass and its fresh mass) and seed mass. Plant material of 10 randomly selected, mature and healthy individuals was sampled during the growing seasons of 2011 and 2012 in the same localities of the Sierra de Guadarrama according to standardized protocols (Cornelissen *et al*., 2003). For each individual, we weighted two fresh and well developed leaves using a microbalance (Mettler Toledo MX5, Columbus, OH; weight uncertainty ±1 µg). The projected surface area of each fresh leaf was estimated with a digital scanner (Epson Perfection 4870) together with Adobe Photoshop CS3 software (Adobe Systems, San Jose, CA). Then the leaves were oven-dried at 60º C for 72 hours and the dry mass measured. Finally, we selected 30 additional individuals by species and collected their seeds. We dried the seeds at environmental temperature for one month and measured the mass of 30 seeds by species using the microbalance (see details above). Select traits represent key components of plant fitness and reflect plant ecological strategies. Plant height is related to the competitive ability for light as well as to the tolerance of environmental stress (Cornelissen *et al.*, 2003; Körner, 2003). SLA and LDMC are linked to leaf economic spectrum of nutrient (Wright *et al.*, 2004), while the first is associated to potential relative growth rate and high photosynthetic capacity (Westoby *et al.*, 2002), the second is related to the toughness and resistance to physical hazard and tends to scaled with 1/SLA (Cornelissen *et al.*, 2003). Seed mass represent the chance to dispersing of a species under a spatio-temporal opportunity (Westoby, 1998). Smaller seeds may be dispersing further away from its progenitors and be produced in a large number while larger seeds have greater availability of resources to survive and develop under environmental hazards (Cornelissen *et al.*, 2003).

Plant Community Assembly

In order to evaluate the role of the freezing resistance traits in the assembly of Mediterranean high mountain communities along environmental gradients we employed the information collected of each of 39 cryophilic grasslands. With the FP and LT_{50} values and species cover in each plot, we estimated the community weighted means (CWMs; Violle *et al.*, 2007; Lepš *et al.*, 2011) and functional diversity for each plot and freezing trait. The CWMs represents the mean trait value of a community considering the relative abundance of each species in each site (weighted by species cover). Subsequently we calculated the functional diversity for each trait separately based on Rao´s quadratic entropy index (RaoQ index; Rao, 1982). This index represents for a certain trait space, the sum of pairwise distances among all possible pairs of species weighted by species abundances and quantifies the functional dissimilarity of dominant species (De Bello *et al.*, 2009). We calculated this metric using a Gower´s distance matrix, as suggested Pavoine *et al.* (2009) and without considering the *F. curvifolia* cover. This species is a genuine ecosystem engineer of this community and is present in all sites with very high covers. This fact could overshadow the functional role of other species.

Statistical analyses

Differences between NT and LT_{50} to determine freezing resistance mechanisms were assessed using *t*-tests, and Mann-Whitney tests when necessary assumptions were not met. The proportion of FT and FA species in each of 39 plots was determined and the change of the corresponding proportion with altitude was established with lineal regression models. Differences in freezing resistance traits (i.e. FP and LT_{50}) among different growth forms were compared with the Kruskal-Wallis rank sum test and a post-hoc Nemenyi test to evaluated honestly significant differences between pairwise growth forms. Similar analyses were carried out to establish the differences between the LT_{50} values grouped by growth forms of our Mediterranean community and the other alpine systems (see above). Relationships among freezing resistance and other plant functional traits were evaluated with Pearson correlations. Finally, the response of environmental (altitude and insolation coefficient) and some community structure predictors (covers of pasture, shrubs and bare soil) on each functional component (CWMs and FD) were modeled by fitting all possible Generalized Linear Models (GLMs). Model and variable selection for each GLM was based on the Akaike information criterion (AIC). In all cases, error distribution considered was Gaussian and the link function identity.

All statistical analysis were made with the software R 2.12.1 (R Development Core Team, 2010); the one way test function of coin package (Hothorn *et al.*, 2008) was applied in Nemenyi test calculations; dbFD function implemented in the FD package (Laliberté & Legendre, 2010; Laliberté & Shipley, 2011) in the case of the community weighted means and functional diversity calculations; dredge function of MuMIn package (Kamil, 2013) to generate the set of GLMs.

RESULTS

Summer freezing resistance of 42 species from Mediterranean high mountain covered an ample range. LT_{50} varied from -18.6 ± 0.2°C in the graminoid *Deschampsia flexuosa* (L.) Trin. (Fam. Poaceae) to -5.1 ± 0.1°C in the case of the hemicryptophyte forb *Coincya monensis* subsp. *cheiranthos* (Vill.) Aedo, Leadlay & Muñoz Garm. in Castrov. & al. (eds.) (Fam. Brassicaceae). Almost a half (47.6%) of them was classified as freezing tolerant, while a 42.9% as freezing avoidant. Only a tiny fraction of 9.5% was sensitive to frost damage. Presence of FA species were higher at low altitudes (around 70%) while proportion of FT plant species increased along altitude ($R^2 = 0.22$, $P = 0.002$). LT_{50} of freezing tolerant species was 4.9 K lower than in the case of freezing avoidant species (Mann-Whitney test, $W = 44.5$, $P < 0.001$). Freezing point (FP) also showed a broad range, from -6.9 ± 0.6°C in leaves of *Koeleria crassipes* Lange to -0.6 ± 0.2°C in leaves of *Biscutella valentina* subsp. *pyrenaica* (A. Huet) Grau & Klingenberg (Table 1). LT_{50} varied among growth forms (Fig. 1) being the hemicryptophyte caespitoses the most freezing resistant group, with an average LT_{50} of 7.2 K and 6.3 K lower than for shrub and hemicryptophyte forbs, respectively. Cushion chamaephytes were ranked in an intermediate position with an average LT_{50} 4.3K higher than for caespitoses (Fig. 1). A similar trend was detected in Austrian Alps although with higher LT_{50} values. Chilean Andes species were generally more resistant than Guadarrama species except in the case of hemicryptophyte caespitoses (Fig. 1). However, extremely resistant cases in these two mountain ranges were quite similar for cushion chamaephytes and hemicryptophyte forbs groups. Australian species were ranked in an intermediate position (Fig. 1). Furthermore, FP of our species followed a similar LT_{50} pattern although only caespitoses and forbs were significantly different.

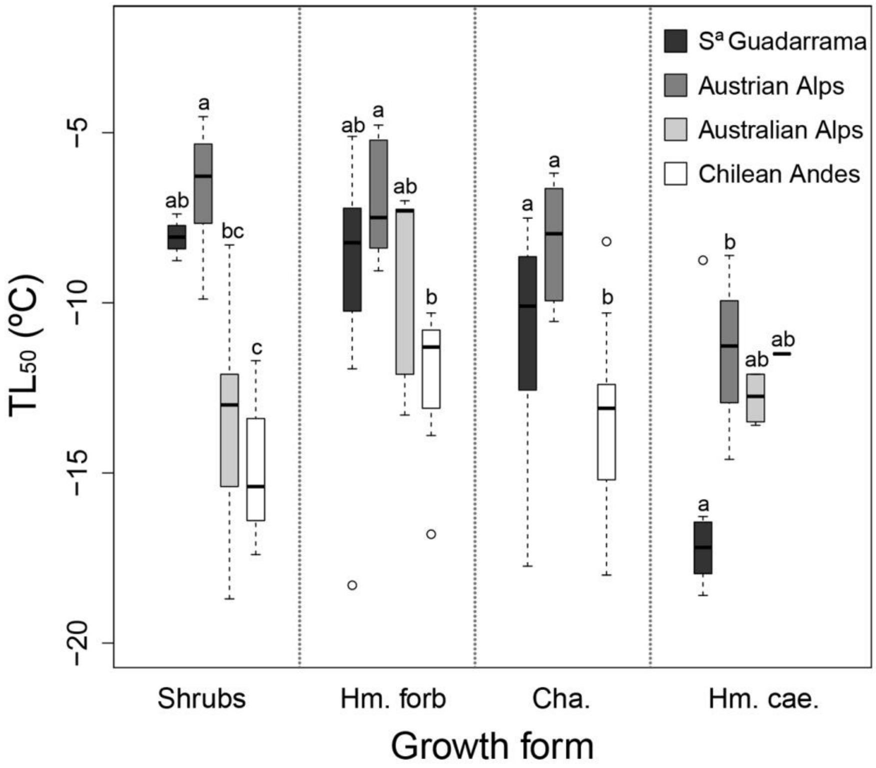

Fig. 1 Freezing temperature damage (LT_{50} - ºC) by growth form in our study system and in three additional alpine plant studies. Shrubs (erect shrubs), Hm. forb (hemicryptophyte forbs), Cha. (cushion chamaephytes) and Hm. cae. (hemicryptophyte caespitoses). For each box plot, median (solid line), interquartile range (box enclosure), variability outside the upper and lower quartiles that is no more than 1.5 times the length of the box away from the box (extreme "*whisker"*) and outliers (black points). Pairs of box plots labeled with different letter above of "*whisker*" differ significantly ($P < 0.05$). Significance was evaluated by a post-hoc Nemenyi test within each growth form.

Among the four considered plant functional traits only LDMC was significantly and negatively correlated with FP for both FA and FT species (Fig. 2A; $R^2 = 0.41$, $P = 0.007$ and $R^2 = 0.24$, $P = 0.03$ respectively) and negatively correlated with LT_{50} in case of FT (Fig. 2B; $R^2 = 0.30$, $P = 0.01$). Moreover, we have not found a significant correlation with plant height for both FP and LT_{50} traits ($r = 0.06$, $P = 0.72$ and $r = 0.18$, P = 0.27 respectively). FS species were not included in the analysis due to low number of plant species within this category.

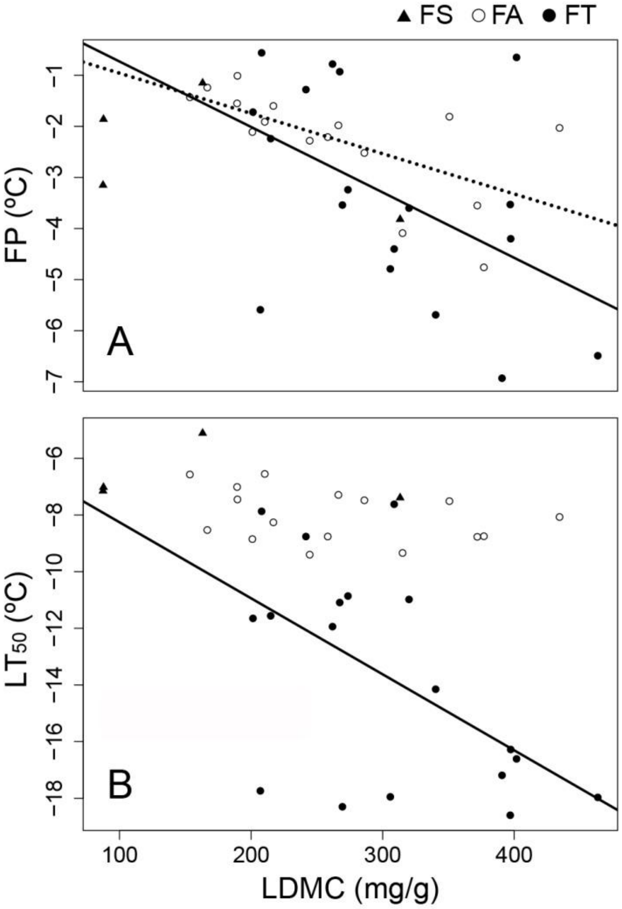

Fig. 2 Relationships between leaf dry matter content (LDMC) with (A) freezing point (FP - ºC) and (B) freezing temperature damage (LT_{50} - ºC), according to freezing resistance mechanism. FT (freezing tolerant; black circles), FA (freezing avoidant; white circles) and FS (freezing sensitive; black triangles). The significant relationship between traits is schematized by different line for each single mechanism considered; FT (solid line) and FA (dashed line).

The community survey showed different responses of FP and LT_{50} with altitude, pasture and shrubs covers when we did not include *F. curvifolia* in the analysis (see Supplementary Data Table S3 for results with *F. curvifolia*). Specifically, the best model to explain CWMs of FP depended negatively on the altitude and positively on the pasture cover ($F_{2\text{-}36} = 5.1$, $P = 0.01$; Fig. 3A, B), while CWMs of LT_{50} was positively explained by pasture and shrubs cover ($F_{2\text{-}36} = 5.0$, $P = 0.01$; Fig. 3B, C). In relation to the Functional Diversity metric (i.e. RaoQ) the selected model included the altitude negatively for both FP ($F_{1\text{-}37} = 9.7$, $P = 0.003$) and LT_{50} ($F_{1\text{-}37} = 4.4$, $P = 0.04$) traits (Fig. 3D).

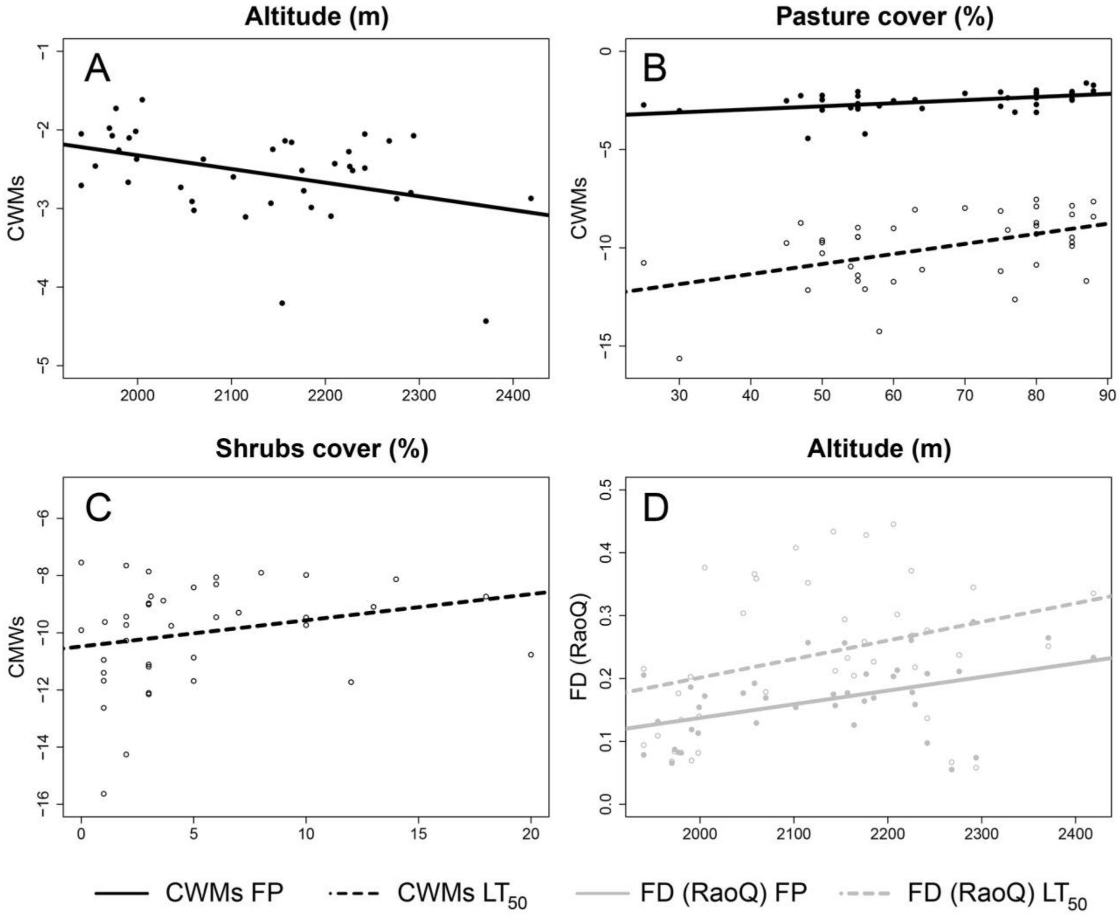

Fig. 3 Scatter plots of community response based on different functional components and the environmental gradients considerate along to 39 plots. The significant relationship between each environmental variable, (A,D) altitude, (B) pasture and (C) shrubs cover, and community weighted means (CWMs – black circles) or functional diversity (FD; grey circles) is represented for freezing point (FP - ºC; solid circles) and freezing temperature damage (LT_{50} - ºC; open circles). Lines represent the regression lineal model slopes.

Table 1. Freezing resistance in leaves of forty two species from Sierra de Guadarrama (Spain) during the growing season. Parameters: NT, ice nucleation temperature; FP, freezing point; LT$_{50}$, temperature producing 50% damage. Values are shown as mean ± SE. Mechanisms: FA, freezing avoidance; FT, freezing tolerance; FS, freezing sensitive. Growth-form abbreviations: Hm cae, hemicryptophyte caespitoses; Hm forb, Hemicryptophyte forb; Cs, cushion chamaephyte; Sh, erect shrub.

Species	Family	Growth form	NT (ºC)	FP (ºC)	LT$_{50}$ (ºC)	Mech.
Adenocarpus complicatus (L.) J. Gay in Durieu	Fabaceae	Sh	-13.32 ± 0.5	-3.82 ± 0.17	-7.39 ± 0.08	FS
Agrostis delicatula Pourr. ex Lapeyr.	Poaceae	Hm cae.	-8.54 ± 0.27	-4.2 ± 0.58	-16.28 ± 0.45	FT
Agrostis rupestris All.	Poaceae	Hm cae.	-8.18 ± 0.4	-4.76 ± 0.5	-8.75 ± 0.08	FA
Alchemilla saxatilis Buser	Rosaceae	Hm forb	-8.58 ± 0.42	-3.55 ± 0.61	-8.77 ± 0.38	FA
Armeria caespitosa (Gómez Ortega) Boiss. in DC.	Plumbaginaceae	Ch	-6.66 ± 0.35	-1.81 ± 0.16	-7.51 ± 0.3	FA
Biscutella valentina subsp. *pyrenaica* (A. Huet) Grau & Klingenberg	Brassicaceae	Hm forb	-5.37 ± 0.48	-0.56 ± 0.18	-7.87 ± 0.33	FT
Campanula herminii Hoffmanns. & Link	Campanulaceae	Hm forb	-8.67 ± 0.45	-2.24 ± 0.62	-11.56 ± 0.8	FT
Coincya monensis subsp. *cheiranthos* (Vill.) Aedo, Leadlay & Muñoz Garm. in Castrov. & al. (eds.)	Brassicaceae	Hm forb	-7.98 ± 0.4	-1.15 ± 0.29	-5.11 ± 0.08	FS
Cytisus oromediterraneus Rivas Mart. & al.	Fabaceae	Sh	-8.89 ± 0.77	-2.21 ± 0.71	-8.76 ± 0.56	FA
Deschampsia flexuosa (L.) Trin.	Poaceae	Hm cae.	-7.76 ± 0.25	-3.53 ± 0.2	-18.6 ± 0.23	FT
Dianthus lusitanicus Brot.	Caryophyllaceae	Ch	-7.94 ± 0.16	-3.24 ± 0.3	-10.86 ± 0.85	FT
Digitalis purpurea subsp *purpurea* L.	Scrophulariaceae	Hm forb	-6.07 ± 0.09	-1.29 ± 0.12	-8.21 ± 0.25	FT
Erysimum penyalarense (Pau) Polatschek.	Brassicaceae	Hm forb	-7.79 ± 0.79	-1.72 ± 0.3	-11.65 ± 0.17	FT
Festuca curvifolia Lag. ex Large	Poaceae	Hm cae.	-10.1 ± 0.67	-6.49 ± 0.74	-17.97 ± 0.43	FT
Gentiana lutea L.	Gentianaceae	Hm forb	-7.6 ± 0.49	-0.93 ± 0.44	-11.09 ± 0.32	FT
Herniaria glabra L.	Illecebraceae	Hm forb	-8.08 ± 0.31	-4.18 ± 0.16	-8.95 ± 0.31	FA
Jasione crispa subsp. *crispa* (Pourr.) Samp.	Campanulaceae	Ch	-6.06 ± 0.22	-1.28 ± 0.15	-8.76 ± 0.41	FT
Juniperus communis subsp. *alpina* (Suter) Célak	Cupressaceae	Sh	-7.27 ± 0.5	-2.03 ± 0.31	-8.07 ± 0.07	FA
Jurinea humilis (Desf.) DC.	Asteraceae	Hm forb	-6.68 ± 0.41	-1.98 ± 0.37	-7.29 ± 0.78	FA
Koeleria crassipes Lange	Poaceae	Hm cae.	-11.27 ± 0.69	-6.93 ± 0.63	-17.19 ± 0.61	FT

Leucanthemopsis alpina (L.) Heywood	Asteraceae	Hm forb	-8.46 ± 0.54	-1.6 ± 0.25	-8.26 ± 0.25	FA
Linaria saxatilis (L.) Chaz.	Scrophulariaceae	Hm forb	-7.91 ± 0.51	-1.91 ± 0.2	-6.55 ± 0.96	FA
Luzula spicata (L.) DC. in Lam. & DC.	Juncaceae	Hm cae.	-9.4 ± 0.53	-4.79 ± 0.42	-17.95 ± 0.37	FT
Minuartia recurva (All.) Schinz & Thell.	Caryophyllaceae	Ch	-10.25 ± 0.11	-5.69 ± 0.3	-14.15 ± 0.34	FT
Murbeckiella boryi (Boiss.) Rothm.	Brassicaceae	Hm forb	-6.91 ± 0.33	-1.43 ± 0.25	-6.57 ± 0.58	FA
Nardus stricta L.	Poaceae	Hm cae.	-4.68 ± 0.33	-0.65 ± 0.29	-16.61 ± 0.58	FT
Paronychia polygonifolia (Vill.) DC. in Lam. et DC.	Illecebraceae	Ch	-8.58 ± 0.39	-4.09 ± 0.52	-9.34 ± 0.28	FA
Phyteuma hemisphaericum L.	Campanulaceae	Hm forb	-10.79 ± 0.51	-3.54 ± 0.68	-18.3 ± 0.3	FT
Pilosella castellana (Boiss. & Reut.) F.W. Schultz & Sch. Bip.	Asteraceae	Hm forb	-6.91 ± 0.68	-0.78 ± 0.22	-11.94 ± 0.74	FT
Pilosella vahlii (Froel.) F.W. Schultz & Sch. Bip.	Asteraceae	Hm forb	-8.51 ± 0.21	-2.11 ± 0.39	-8.85 ± 0.07	FA
Rumex acetosella L.	Polygonaceae	Hm forb	-8.51 ± 0.64	-1.55 ± 0.14	-7.01 ± 0.5	FA
Saxifraga pentadactylis subsp. *willkommiana* (Boiss. ex Willk.) Rivas Mart.	Saxifragaceae	Ch	-9.3 ± 0.74	-1.24 ± 0.35	-8.53 ± 0.3	FA
Sedum brevifolium DC.	Crassulaceae	Hm forb	-10.63 ± 0.67	-3.15 ± 0.41	-7.15 ± 0.55	FS
Sedum candollei Raym.-Hamet	Crassulaceae	Hm forb	-8.59 ± 0.35	-1.86 ± 0.47	-7.02 ± 0.51	FS
Senecio boissieri DC.	Asteraceae	Hm forb	-11.03 ± 0.42	-4.64 ± 0.7	-11.14 ± 1.14	FA
Senecio carpetanus Boiss & Reuter	Asteraceae	Hm forb	-8.62 ± 0.66	-1.01 ± 0.31	-7.45 ± 0.14	FA
Silene boryi Boiss.	Caryophyllaceae	Hm forb	-8.43 ± 0.39	-2.28 ± 0.14	-9.4 ± 0.69	FA
Silene ciliata Pourr.	Caryophyllaceae	Ch	-11.93 ± 0.38	-5.59 ± 0.36	-17.74 ± 0.51	FT
Solidago virgaurea subsp. *fallit-tirones* (Font Quer) Rivas Mart.	Asteraceae	Hm forb	-7.93 ± 0.62	-2.52 ± 0.39	-7.48 ± 0.63	FA
Taraxacum sp.	Asteraceae	Hm forb	-7.16 ± 0.41	-2.13 ± 0.3	-9.31 ± 0.3	FT
Thymus praecox subsp. *penyalarensis (Pau) Rivas Mart.*	Lamiaceae	Ch	-8.39 ± 0.41	-3.6 ± 0.45	-10.98 ± 0.94	FT
Veronica fruticans subsp. *cantabrica* M. Laínz	Scrophulariaceae	Hm forb	-9.49 ± 0.3	-4.4 ± 0.14	-7.62 ± 0.03	FT

DISCUSSION

Summer freezing resistance is critical for plant life in alpine habitats and an important determinant of the structure and functioning of these plant communities. However, multi-species studies dealing with this resistance are relatively scarce (but see Taschler and Neuner 2004; Bannister *et al*., 2005; Sierra-Almeida *et al*., 2009; Sierra-Almeida *et al*., 2010; Venn *et al*., 2013). Our results showed the importance of freezing summer traits in the organization and assembly of Mediterranean high mountain communities.

We found a wide range of summer freezing resistance strategies among Mediterranean high mountain species (LT_{50} range from -5.1 to -18.6°C; Table 1). More than 60% of studied species are able to resist temperatures below -8°C, the absolute minimum air temperature during the growing season in the study area at least for the last 40 years (Supplementary Data Table S1). This implies that the majority of them have the ability to cope with freezing events during summer seasons. Median LT_{50} of these species (-8.8ºC) were higher when compared to other Mediterranean mountains (i.e. median LT_{50} -13.2ºC in Chilean Andes; Sierra-Almeida *et al*., 2010) or other temperate mountains (i.e. median LT_{50} -12.45ºC in Australian Mountain; Venn *et al*., 2013), but they were similar to that reported for Austrian alpine species (i.e. median LT_{50} -8.1°C; Taschler and Neuner 2004). However, the wide range and especially the extreme values of LT_{50} are similar to those of other reported species from mountains located in dry climates (Fig. 1). For example, plants resist temperatures from -7.5 to -16ºC in East Pamirs (Tyurina, 1957 in Sakai and Larcher, 1987), from -4.7 to -20°C in the Doña Ana Mountains, Northern Chile (Squeo *et al*., 1996), and from -8.2 to -22°C in the Mediterranean type climate Chilean Andes (Sierra-Almeida *et al*., 2010). Taken together, our findings suggest that summer drought resistance of Mediterranean high mountains locate these species among the most resistant of all the known alpine plant species.

Freezing point (FP) also showed an ample variability among our species and was positively correlated with LT_{50} ($r = 0.30$, $P < 0.001$). FP depends on some properties of the plant tissues and may vary according to the cell sap concentration and/or the accumulation of water-binding substances inside the cell (Sakai & Larcher, 1987). Several studies have reported that water-soluble carbohydrates content could depress FP, and their accumulation is positively related to drought tolerance (Streeter *et al.*, 2001; Merchant *et al.*, 2006; Monson *et al.*, 2006). Moreover, Sierra-Almeida *et al.* (2009) reported an intra-seasonal decrease of FP in plants from Mediterranean Chilean Andes that could be attributed to an increase of drought intensity towards the end of the growing season especially at lower elevations. In our case, the coincidence of the most freezing resistant species with the lowest FP values suggest that drought related traits could explain at least partially the high tolerance to freezing events of Mediterranean high mountains species.

Freezing resistance mechanisms were evenly distributed among the studied species, with a 47.6 and 42.9% of them being classified as freezing tolerant (FT) and freezing avoidant (FA) respectively. FT mechanism is also predominant among Mediterranean Chilean Andes species, especially at higher altitudes, where frost events are more severe and of longer duration (Squeo *et al.*, 1996; Sierra-Almeida *et al.*, 2009; 2010). Interestingly the proportion of FA to FT changed with the altitude. Given that this is an operational classification, more proportion of FA at low altitudes is mainly tied to a decreased in NT, which depends on water status cells and its structure (e.g. size, cell wall width; Larcher, 2003). Therefore, the highest intensity of drought at low altitudes could be affecting water status, with less water in the tissues to freeze, and forcing the type of mechanism (Sierra-Almeida *et al.*, 2009).

Freezing resistance seems to be also related to the growth strategy with the highest and the lowest values of FP-LT_{50} varying from hemicryptophyte forbs to caespitoses respectively. A similar pattern was found in the Alps (Fig.1; Taschler and Neuner, 2004), where graminoids were also the most frost-tolerant species (see also Körner, 2003; Bannister, 2005) . The biogeographic relatedness of both floras sharing close relatives of the same genus and species widely distributed in the Holarctic world (e.g. *Juniperus*, *Poa*, *Nardus* or *Silene*) could be possible explanations. Worth to note that the resistance of our graminoids is higher than in other mountains (Taschler & Neuner, 2004; Sierra-Almeida *et al.*, 2009; Venn *et al.*, 2013), probably due to high leaf toughness and the sclerophyllous habit which are known traits related to drought resistance and typical of Mediterranean plants (Gullo & Salleo, 1988).

Ecological performance under stressful conditions is determined by a combined response of several traits (MacGillivray *et al.*, 1995; Violle *et al.*, 2007). Consequently, a correlation between frost resistance and other functional traits is expected due to a certain phenotypic integration. For instance Squeo *et al.* (1991) showed a negative correlation between plant height and frost-resistance in Puna-type Andean plants. Our results concur with those of other Mediterranean type mountains (Sierra-Almeida *et al.*, 2010) showing no correlation with height; furthermore LT_{50} values of our highest plants which are some shrubs were among the lowest of our community. Thus, this global pattern between height and frost-resistance does not seem to occur in Mediterranean ecosystems probably due to extreme summer drought (Sierra-Almeida *et al*., 2010). We also considered other key functional traits as potential predictors of frost resistance, but only leaf dry matter content (LDMC) showed a significant correlation with FP and LT_{50}. Both FT and FA species showed higher LDMC at lower FP values (more negative temperatures). This trend may be due to an osmotic adjustment which is similar to the response to drought. Plants suffering such conditions, freezing or water shortage, may synthesize and accumulate solutes and/or thicken and waterproof the cell walls, increasing the LDMC values and decreasing the water content in intercellular spaces. Thus leaves with high LDMC, often rigid and with more structural material (Cornelissen *et al.*, 2003), could develop a greater negative turgor pressures providing greater frost resistance parallel to water stress tolerance (resistance to freeze dehydration; Anisko and Lindstrom, 1996). However leaves of FA species with greater LDMC values did not give more freezing resistance (LT_{50} was not correlated with LDMC) contrarily to FT species where this relationship was found. This concurs with the fact that this freezing evasion is a resistance mechanism under moderate and short duration freezing events. Therefore our findings linking freezing and drought resistance traits suggests that the latter may be a pre- or co-adaptatitive condition in some xeric mountains species which will provide greater resistance in these mountain habitats (Sakai & Larcher, 1987).

At the community level, freezing resistance is related to some environmental and structural factors (i.e. altitude, shrubs and pasture covers) suggesting FP and LT_{50} represent a response to critical habitat filters. As we hypothesized freezing resistance values weighted by species abundances in each plot dropped along altitude (Fig. 3A). The entrance of elements more resistant (e.g. *Agrostis rupestris* All., *Phyteuma hemisphaericum* L. or *Veronica fruticans* subsp. *cantabrica* M. Laínz) and cover increment of some of them (e.g. *Minuartia recurva* (All.) Schinz & Thell. or *Silene* *ciliata* Pourr.) at higher altitudes could be responsible of this decline. In addition, plots with higher pasture and shrubs covers are characterized by higher mean values of LT_{50} and FP (Fig. 3B, C). It is known that cushion plants provide further *secure sites* for other plant species in alpine habitats (Kikvidze *et al.*, 2005; Cavieres *et al.*, 2006). Their low stature, dense canopy, and compact form allow them to decouple their microclimate from the surrounding environment, mitigating the effect of low temperatures and drought, enhancing the survival of other species (Cavieres *et al.*, 2007). Therefore, plants with relatively low freezing resistance may reach altitudes where freezing events are hard by interacting with other plant species. Most interesting was the response of functional diversity of freezing resistance traits along gradients (Fig. 3D). The decreased in functional diversity for FP and LT_{50} at low altitudes suggests local coexistence of species with similar freezing trait values. This finding would be unexpected under the extended idea that in alpine habitats an increase of stress with altitude is the norm (Körner, 2007) and functional diversity is lower when stress increases (Weiher and Keddy, 1995; Cornwell and Ackerly, 2009; but see Spasojevic and Suding, 2012). Two not exclusive explanations are possible. First, since our mountain range is not very high (only 2419 m), the summit could not constitute a real freezing edge and the expected drop with altitude not found (Taschler & Neuner, 2004; Sierra-Almeida *et al.*, 2009). Second, summer drought which is a typical feature of Mediterranean mountains increases as altitude descends (Giménez-Benavides *et al.*, 2007) and this constraint would be more relevant for plant life than the increase of severity with altitude (Cavieres *et al.*, 2006; Schöb *et al.*, 2013).

To our knowledge this is the first time in which the response to freezing summer events is shown to be relevant in the assembly of alpine communities since a linear trend of the community weighted mean and functional diversity with these freezing traits were found. Moreover, the low proportion of FS among studied species and increase of FT species along altitude support the idea that this physiological capability is necessary to surpass this primary abiotic filter. In addition, our results also showed that the coincidence of an opposite gradient with altitude of water shortage in Mediterranean high mountains make the community and species responses extremely complex. So, leaf dry matter content of different species was positively correlated with FP and LT_{50} for tolerant species, suggesting a link between water shortage and freezing resistance exists. This probably causes that graminoids, with higher LDMC values, were the most resistant plant group both in our system and also in other xeric mountains. Our results reinforce the idea that summer drought at which Mediterranean plant species are exposed could explain the high resistance to freezing events in these high mountain specialists.

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SUPPLEMENTARY MATERIAL

Table S1. Climate information for the study region from 1946 to 2011 (Navacerrada Pass weather station; 40°47' 35" N, 4°0' 38" W; 1896 m a.s.l.). Source: *Agencia Estatal de Meteorología - Ministerio de Agricultura, Alimentación y Medio Ambiente* of Spain.

	Anual	Growing season	May	June	July	August	Sep
Mean air temperature (°C)	6.48	12.88	7.05	12.29	16.42	16.20	12.43
Mean minimum air temperature (°C)	2.77	8.18	3.09	7.65	11.15	10.96	8.06
Absolute minimum air temperature (ºC)	-20.30	-8.00	-8.00	-3.40	0.00	0.20	-3.00
Mean maximum air temperature (°C)	10.18	17.56	11.00	16.90	21.67	21.42	16.79
Absolute maximum air temperature (ºC)	31.80	31.80	25.40	29.00	30.80	31.80	30.80
Precipitation (mm/month)	109.86	64.61	128.52	69.11	24.19	27.55	71.33
Mean days of rain	67.45	33.17	8.34	8.24	4.42	5.20	8.40
Mean days of snow	72	6	5	1	0	0	0
Mean days of hail	5	7	3	2	1	1	1
Insolation (hours/day)	6.07	8.99	6.97	9.21	11.16	10.40	7.13
Insolation (%)	48.09	63.52	48.02	61.26	75.58	75.41	57.00
Days/month with air T < 0ºC	12	2	8	1	0	0	1

Table S2. Additional plant functional traits. Means and sd (standard deviation) values of the four functional traits for most species sampled in thermal analyses.

Species	Hmax (mm)		SLA ($mm^2 \cdot mg^{-1}$)		LDMC ($mg \cdot g^{-1}$)		Seed mass (µg)	
	mean	sd	mean	sd	mean	sd	mean	sd
Adenocarpus complicatus	789.4	113.9	10.2	3.7	313.2	40.3	$4.4 \cdot 10^4$	4703.2
Agrostis delicatula	20.5	6.8	11.4	2.0	397.3	44.5	63.7	13.8
Agrostis rupestris	37.3	9.4	15.9	1.1	376.9	16.9	52.7	18.3
Alchemilla saxatilis	49.8	21.1	13.4	2.0	371.9	28.9	384.4	116.6
Armeria caespitosa	16.2	4.3	15.1	2.2	350.7	34.9	641.7	94.0
Biscutella valentina subsp. *pyrenaica*	33.4	10.5	11.8	2.1	208.0	20.3	1202.9	231.7
Campanula herminii	19.9	5.6	24.2	2.5	214.9	17.6	60.4	5.1
Coincya monensis subsp. *cheiranthos*	37.2	13.3	8.3	8.8	163.1	23.0	1428.4	363.2

Table S2. Cont.

Cytisus oromediterraneus	502.5	120.8	14.3	2.2	258.2	32.1	$1.1\cdot10^4$	1526.1
Deschampsia flexuosa	27.6	9.4	7.7	1.2	396.9	30.7	617.1	130.0
Dianthus lusitanicus	33.6	10.9	8.9	1.3	273.6	18.0	1038.2	203.5
Erysimum penyalarense	30.6	11.4	20.1	4.3	201.4	33.8	1428.4	363.2
Festuca curvifolia	32.4	9.7	4.3	0.6	463.5	40.4	681.1	146.5
Gentiana lutea	182.0	60.5	8.9	1.0	267.3	14.7	964.7	243.6
Jasione crispa subsp. *crispa*	15.0	4.5	18.4	3.7	241.6	27.6	49.6	5.2
Juniperus communis subsp. *alpina*	233.1	85.0	6.5	1.1	434.4	46.6	$1.3\cdot10^4$	$1.2\cdot10^4$
Jurinea humilis	7.0	3.3	10.1	1.5	266.3	34.9	7019.8	1239.3
Koeleria crassipes	25.9	3.8	12.6	0.6	390.7	11.0	236.7	37.1
Leucanthemopsis alpina	18.0	5.7	17.3	2.9	216.9	25.2	122.3	23.7
Linaria saxatilis	17.7	3.4	21.5	4.2	210.4	22.4	64.5	10.0
Luzula spicata	44.4	9.7	13.3	2.6	305.8	30.2	253.6	34.1
Minuartia recurva	16.7	7.0	17.2	2.8	340.2	47.3	248.5	37.9
Murbeckiella boryi	19.2	14.5	24.2	5.1	153.4	26.8	121.2	12.8
Nardus stricta	85.3	15.6	6.8	0.8	401.7	17.2	239.0	29.1
Paronychia polygonifolia	20.0	7.9	18.4	3.2	315.1	44.2	351.5	44.7
Phyteuma hemisphaericum	22.2	6.6	18.1	2.3	269.4	17.4	73.0	12.4
Pilosella castellana	22.3	13.4	13.8	1.3	261.9	26.2	181.2	18.4
Pilosella vahlii	21.9	8.3	17.1	2.8	201.0	28.2	181.2	18.4
Rumex acetosella	43.8	19.6	10.6	1.5	189.3	24.4	335.6	65.1
Saxifraga pentadactylis subsp. *willkommiana*	38.3	15.9	6.6	7.1	166.7	25.6	45.7	7.0
Sedum brevifolium	13.1	5.4	10.3	2.2	87.5	20.0	68.4	8.9
Sedum candollei	12.7	3.0	19.3	2.7	87.8	9.8	21.6	2.4
Senecio carpetanus	313.8	89.6	10.3	1.5	189.6	29.3	2455.6	357.4
Silene boryi	34.4	7.2	14.3	2.4	244.5	24.4	778.3	192.7
Silene ciliata	19.6	5.1	14.1	1.9	207.0	24.2	348.9	54.0
Solidago virgaurea subsp. *fallit-tirones*	23.5	6.0	12.3	1.0	286.1	16.5	862.0	159.4
Thymus praecox subsp. *penyalarensis*	21.1	4.8	14.9	1.7	320.0	27.4	197.5	37.5
Veronica fruticans subsp. *cantabrica*	17.1	5.2	12.9	1.8	308.7	29.6	157.1	21.7

Hmax, maximum plant height; SLA, specific leaf area; LDMC, leaf dry matter content

Table S3. Model selection, variables and parameters without and with *F.curvifolia* cover consideration, for each functional component (CWMs – FD) and freezing resistance trait (FP – LT_{50}).

Without *F. curvifolia* cover

	Alt.	Ins.	Pas.r	Shr.	AICc	R^2	F	P
CWMs- FP	-0.001		0.01		63.4	0.22	5.1	0.01
CWMs-LT_{50}			**0.04**	0.11	155.4	0.22	5.0	0.01
FD-FP	**$2.1 \cdot 10^{-4}$**				-111.84	0.21	9.7	0.004
FD-LT_{50}	**$2.9 \cdot 10^{-4}$**				-56.9	0.11	4.4	0.043

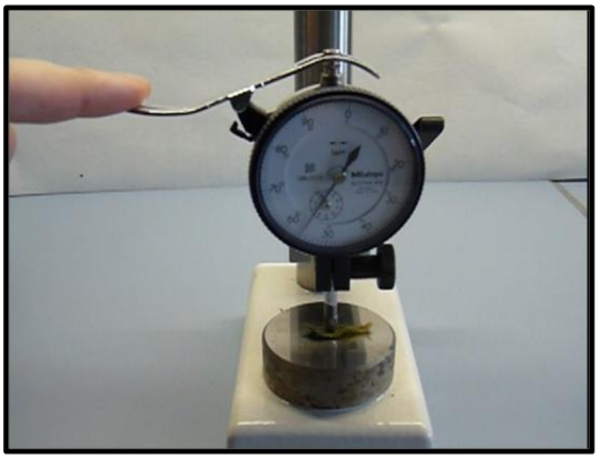
With *F. curvifolia* cover

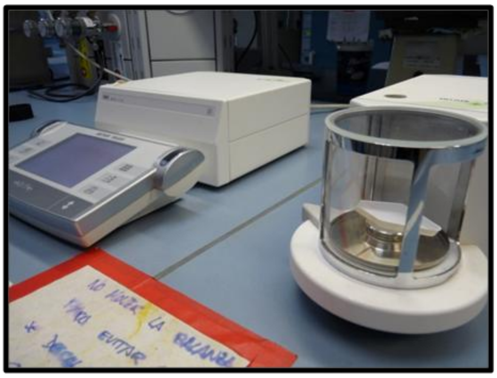
	Alt.	Ins.	Pas.	Shr.	AICc	R^2	F	P
CWMs-FP			**-0.008**	**0.035**	35.68	0.3	7.78	0.002
CWMs-LT_{50}				**0.1**	82.7	0.36	21.03	<0.001
FD-FP	**$9.3 \cdot 10^{-5}$**		**0.0018**		-157.71	0.41	12.41	<0.001
FD-LT_{50}	**$-6.4 \cdot 10^{-5}$**	**-0.087**	$3.2 \cdot 10^{-4}$	**-0.003**	-209.49	0.6	110.7	<0.001

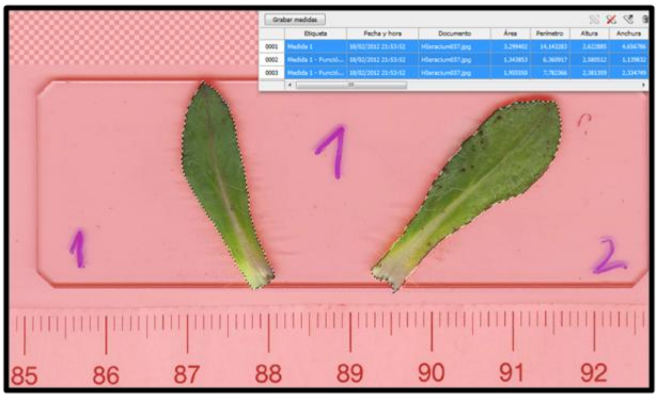
Alt., altitude (m); Ins, insolation coefficient; Pas, pasture cover (%); Shr., shrubs cover (%). Values of each variable represent the estimated slope coefficient. Only those variables with values were present in the model. Values in bold represent estimated coefficients significantly different from zero.

II

Plant trait variation along an altitudinal gradient in Mediterranean high mountain grasslands: controlling the species turnover effect







Pescador, D.S., de Bello, F., Valladares, F. and Escudero, A.

En revisión en Plos One

ABSTRACT

Assessing changes in plant functional traits along gradients is useful for understanding the assembly of communities and their response to global and local change drivers. However, these changes may reflect the effects of species turnover and intra-specific variability. In order to determine the relevance of the latter, trait variation must be assessed under minimal effects of species turnover. Nine sampling sites were established along an altitudinal gradient in a Mediterranean high mountain grassland community with low species turnover (Madrid, Spain, 40º47´ N; 3º58´ W; 1940 m – 2419 m a.s.l.). Nine functional traits were measured in ten plant species at each site, for a total of eleven species across all sites. The relative importance of two sources of variability (within/between site and intra-/inter-specific functional diversity) and altitudinal response of individual species and community were explored. Effects of turnover and intra-specific variability on FD response to altitude were also assessed. We found a weak individual species response to altitude, although in some cases, individuals were smaller and leaves were thicker at higher elevations. This lack of species response was most likely due to greater within- than between-site species variation. At the community level, functional diversity partitioning showed low, albeit non-negligible, intra-specific differentiation, except for traits linked to nutrient content. Contrary to existing evidence, inter-specific functional diversity increased with altitude for four leaf traits (Specific Leaf Area, Leaf Dry Matter Content, $\delta 13C$ and $\delta 15N$), suggesting trait convergence between species at lower elevations. Although micro-environmental conditions seem to be more important than altitude in determining the functional response of each species, typical Mediterranean water shortage at lower elevations may have a stronger environmental filtering effect than colder temperatures at higher altitudes. Our study illustrates the importance of considering intra-specific trait

variability in the study of community trait assembly, provided the turnover effect is controlled.

INTRODUCTION

Knowing how plant fitness and phenotypic expression change along environmental gradients is essential for understanding the response of plant communities to global change drivers [1]. In this sense, patterns of plant functional trait variation provide inform not only on community structure but also on population dynamics and the mechanisms affecting ecosystem functioning [2-4]. Variation in individual responses to changes in plant functional traits within and/or between sites and/or species with environmental gradients may reflect deterministic processes of community organization.

Numerous studies have found variation in the distribution of plant traits along different environmental gradients and spatio-temporal scales. In the case of a well known severity gradient like altitude [5] variation has been reported within species [6]. Other studies have focused on vicariant species [7], found differences between lowland *versus* highland species [8] and some have directly evaluated responses in complete plant communities along altitude [4, 9, 10]. As conclusion, functional responses have been documented along altitudinal gradients, although many aspects have yet to be explored. For instance, how to scale up this known functional variation to the community level still remains a challenge. Shifts in trait distribution along altitudinal gradients can be regarded as a consequence of abiotic change or competitive exclusion [11]. Environmental would act as a primary filter selecting individuals and species with a trait composition capable of tolerating particular conditions [12, 13]. If so, certain trait would be exacerbated as conditions become harsher, increasing intra- and inter-specific trait similarity [14, 15].

Although a new paradigm in which the importance of within site and intra-specific trait variation is arising [9, 16-19], we are far from reaching a unanimous conclusion

[10, 20, 21]. Trait variance partitioning and the consideration of intra-specific variability depends on the research question, trait plasticity, the spatial-temporal scale of the study (e.g. length of environmental gradient) and the considered species (see, [16]). One question that remains unanswered is the relative effect of changes in species composition (i.e. turnover) vs. changes in intra-specific trait variability along altitudinal gradients [22]. Previous studies have suggested that intra-specific effects are more important in short environmental gradients, where changes in species composition are small [23]. Unfortunately, most studies that have explored trait shifts along environmental gradients were based on changes in mean trait values caused by species turnover (but see [10]). Thus, conclusions are not straightforward, and patterns in functional traits at the community level are mainly due to species turnover and not to changes within plants in the community and intra-specific variation [24].

Bearing these challenges in mind, we focused on intra- and inter-specific plant functional shifts within and between sites along a large altitudinal gradient within the community (i.e. low species turnover). This implies that a strong habitat filter is acting and only certain species can coexist, even though the community thrives under very different climate conditions. Adjustment to varying local conditions should mainly rely on intra-specific variation between sites, because the entrance of new species is not feasible and the best trait combination would be selected at each site. With this idea in mind we studied the spread of trait distributions in a grassland area that occurs far above the timberline and extends along a large altitudinal gradient. Mediterranean high mountain communities are valuable models for assessing the spread of trait distributions [25], since plant species deal with two opposing severity gradients: a moisture gradient from high to low altitude and a cold temperature effect which increases to the summit (Figure S1). We expect the length and intensity of stress gradient define the importance of inter- *versus* intra-specific plant trait variation, whereas gradient direction identifies the most stressful conditions where the greatest trait similarity should occur. However, since opposite gradients could be operating simultaneously and species composition is similar across our community, some of the functional components could remain invariable.

In this study, we aimed to answer the following questions: (i) How do mean trait values and coefficients of variation of each species respond to shifts in environmental conditions? (ii) How important is within-site and between-site partitioning for each species and functional trait? iii) What is the relevance of intra-specific *versus* inter-specific functional components (i.e. FD) and how do they change along the studied altitudinal gradient? and (iv) What is the relative contribution of species turnover and intra-specific variation on the response of FD to altitude? We considered information of nine plant functional traits under two sources of variability (within/between sites and intra-/inter-specific) to evaluate the effect of altitude on functional responses in a plant community.

MATERIALS AND METHODS

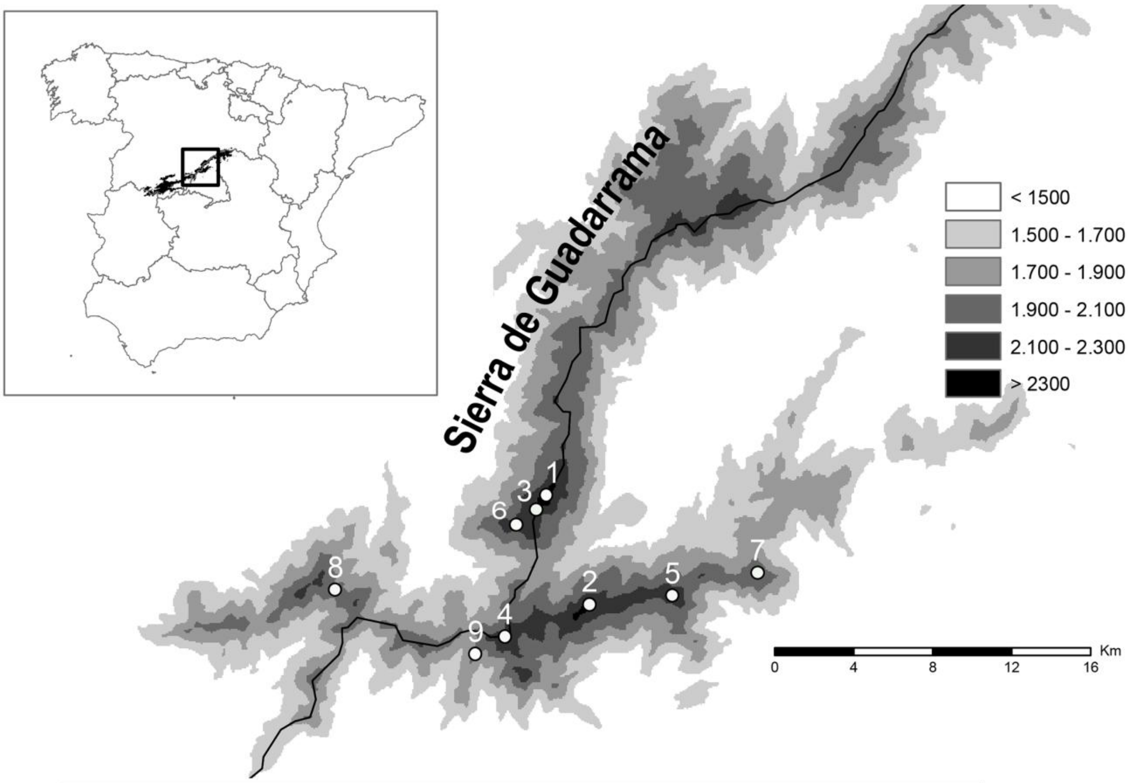
Ethics Statement

Permission for field sampling was obtained from the Dirección General de Medio Ambiente de Madrid. This study did not involve endangered or protected species.

Sites and species selection

In the summer of 2011, we selected 9 sites covering the whole altitudinal range of a *Festuca curvifolia* Lag. ex Lange grassland community, which is located above the tree-line in the Sierra de Guadarrama (40º46´39´´ to 40º51´8´´ N; 3º49´44´´ to 4º4´59´´W; 1940 m – 2419 m a.s.l.; Figure 1). This mountain system is located in central Spain, 70 km north of Madrid. The climate is Mediterranean, with average annual temperature and precipitation values of 6.4 ºC and 1350 mm, respectively (Navacerrada Pass weather station; 40° 47' N, 4° 0' W; 1894 m a.s.l.). Due to intense summer drought, summer precipitation only accounts for a small fraction of total annual precipitation (<10%). Stunted Scots pines (*Pinus sylvestris* L.) form a discontinuous timberline between 1900 and 2100 m interspersed in a shrubby matrix formed by *Cytisus oromediterraneus* Rivas Mart. & al. and *Juniperus communis* L. subsp. *alpina* (Suter) Célak. Our pasture-like community extends above this limit, forming high mountain community islands on the highest summits. This community is dominated by several creeping chamaephytes and caespitosous grasses with a biphasic structure in which plants are clumped in a bare ground matrix.

One sampling plot (quadrats 20 x 20 m) was established at each of the 9 sites, trying to minimize variation in slope, orientation and rockiness. In each plot, we identified the set of the most abundant species, representing over 80% total cover. This set was comprised of 10 species at most sites, and a total of 11 species across all sites, showing that species composition between sites was very similar (Table S1). At each site, 10 random individuals of each species were sampled.



1	Peñalara (2419 m)	2	Cabeza de Hierro (2371 m)	3	Hermana Mayor (2294 m)
4	Bola Oeste (2242 m)	5	Asomate-Hoyos (2225 m)	6	Citores (2157m)
7	La Najarra (2102 m)	8	Collado Tirobarra (1980 m)	9	Navacerrada (1940 m)

Fig. 1. Location of area studied. Distribution of the 9 sites (white dots) sampled in the Sierra de Guadarrama (Spain) and their corresponding altitude.

Different phylogenetic groups of species were sampled, including a coniferous shrub (*Juniperus comunis* subsp. *alpina*), three graminoids (*Agrostis delicatula* Pourr. ex Lapeyr.; *Deschampsia flexuosa* (L.) Trin.; *Festuca curvifolia*), four cushion chamaephytes (*Armeria caespitosa* (Gómez Ortega) Boiss. in DC.; *Jasione crispa* subsp. *crispa* (Pourr.) Samp.; *Minuartia recurva* (All.) Schinz & Thell s.l.; *Silene ciliata* Pourret) and three forbs species (*Pilosella vahlii* (Froel.) F.W. Schultz & Sch. Bip.;

Jurinea humilis (Desf.) DC.; *Senecio pyrenaicus* L. in Löfling). Among these species, we found elements with a wide range of distribution together with some narrow endemics (*F. curvifolia*), Mediterranean high mountain specialists (*J. crispa* subsp. *crispa*) and arctic alpine plants (*D. flexuosa*).

We measured the altitude and orientation of each plot using a GPS (Garmin Colorado-300) and the slope using a clinometer (Silva Clinomaster CM-360-%, LA). Orientation and slope values were used to estimate the insolation coefficient following Gandullo´s method [26, 27]. We sampled species richness in the plot and established five random subplots (2.4 x 2.4 m) to visually estimate species, soil and rock cover. Finally, we randomly collected 5 soil samples (5 cm of diameter, 10 cm depth) in three different microhabitats in each plot: bare ground, vegetated patches dominated by grasses, and shrub areas. Soil samples were sieved (2 mm mesh) and air-dried for 1 month. We then estimated soil organic carbon (SOC) by colorimetry after oxidation with $K_2Cr_2O_2$ and H_2SO_4 [28] and total N on a SKALAR++ San Analyzer (Skalar, Breda, The Netherlands) after digestion in H_2SO_4 and Kjedahl´s catalyst.

Measurement of traits

We measured nine functional traits on the sampled individuals (10 individuals per species and site). Plant size (SI) was estimated using canopy area projection (cm^2) as a surrogate of accumulated resources, adjusting an ellipsoid shape:

$$SI = \pi \cdot {}^{D}/_{2} \cdot {}^{d}/_{2}$$

where *D* represents the largest diameter and *d* the smallest perpendicular. Plant height (Hmax, distance from the ground to the highest photosynthetic tissues) is related to competitive vigor and stress tolerance [29]. The leaf economic spectrum was represented by specific leaf area (SLA; ratio of fresh leaf area to dry leaf weight) and leaf dry matter content (LDMC; dry leaf weight divided by fresh saturated leaf weight). SLA is related to potential relative growth or relative photosynthetic rates, while LDMC is related to toughness and resistance to physical hazards and tends to scale to 1/SLA [29]. We weighed 2 fresh well-developed leaves per individual using a microbalance (Mettler Toledo MX5, Columbus, OH; weight uncertainty ±1 µg). Projected surface area was estimated with a digital scanner (Epson Perfection 4870) and Adobe Photoshop CS3 software (Adobe Systems, San Jose, CA). The leaves were then oven-dried at 60 ºC for 72 hours, and dry mass was measured. We also estimated leaf thickness using a dial thickness gauge (Mitutoyo Co., Aurora, IL, USA). This trait is related to resource acquisition and is often correlated to SLA and LDMC [30]. Finally, leaf carbon concentration (LCC), leaf nitrogen concentration (LNC) and carbon and nitrogen isotope ratios of organic material (δ13C and δ15N) were measured in a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). These two isotopes are interesting traits, because δ13C is linked to water-use efficiency (drought tolerance), and δ15N reveals the net effect of multiple processes, such as mycorrizal associations, dynamics in atmospheric/soil N-sources or changes in internal N cycles [31].

Data analyses

First, we conducted a principal components analysis by using a matrix with each individual and trait value (880 individuals x 9 traits) to determine the global pattern and relationships among all plant traits and species.

We then built a series of models to understand the response and variation of each functional trait and species along different environmental gradients. We built linear mixed-effect models for each plant trait, considering altitude as a fixed factor and site as a random factor. Sites were considered a random factor because a certain level of dependence between all the individuals at a site is expected. This approach is conservative since sites are located along altitude. Hence, part of the variation along altitude should be brushed by this random effect. We developed additional mixed-effect models with other fixed factors at the site level, such as insolation, soil properties (i.e. SOC and NT), species richness and total grass cover (see above for details). We also built linear models where the dependent variable was the coefficient of variation of each plant trait per species, considering altitude as a fixed effect. These models verify changes in variability within species along the gradient.

Our experimental design (10 individuals per species and site) allowed us to survey within-site and between-site variance partitioning fitting a one-way ANOVA for each trait and species, where site was the independent variable [32]. Based on the Sum of Squared decomposition, $SS_{Total} = SS_{site} + SS_{residuals}$, the first term corresponds to total variability; $SS_{site,}$ to between-site variability; and $SS_{residual,}$ to unexplained variability which would be assigned to within-site variability.

At the community level, we estimated two functional metrics. First, we estimated community weighted mean values at each site (CWMs, [22, 33]) to explore community shifts in plant traits along altitude. CWMs represent the mean trait value of a community considering the relative abundance of each species at a specific site (measured here as species cover). We used two CWM values following Lepš *et al*. approach [22]; using mean trait value measured for each species at a given site (CWMp) or using mean value across all sites (CWMf):

$$CWM_p = \sum_{i=1}^{S} p_i \, x_{i\_plot}$$

$$CWM_f = \sum_{i=1}^{S} p_i \, x_i$$

where S is the number of species in the plot, p_i is the cover of the i-th species in a given plot, x_{i_plot} is the trait value of the i-th species for the considered plot and x_i is the trait value of the i-th species for all plots where the species is found. This approach identifies the causes of changes in CWMs across a gradient, which come from three resources of variability: intra-specific trait variation, changes in species composition (intentionally low here) and the covariance between them [22]. We carried out a paired t-test to determine if the two CWMs were different. Besides we calculated functional divergence (FD) at each site as a component of functional diversity. Functional divergence represents trait dissimilarity weighted by species relative abundance at each site [34], which was estimated with the Rao quadratic entropy metric [35]. This metric performs the sums of all possible trait pairwise dissimilarities between species weighted by relative abundance. Subsequently we divided the functional diversity of each site into intra-specific and inter-specific components. See equation 3 in [36] for details. This implementation was similar to the decomposition of the Rao index into with- and among samples [37]. The response of each functional metric to altitude (i.e. CWMs and FD) was evaluated using linear models. Finally, as for CWMs, FD values were computed using site-specific values (FDp) or averaged values across all sites (FDf) to evaluate the importance of intra-specific variation and/or species turnover effects along altitude [36]. Variation in FDp results from intra-specific and species turnover changes, while FDf is only dependent on species turnover, because each individual species has the same average value at all sites. Consequently, the effect of intra-specific trait variability is due to differences between FDp and FDf. To quantify the relative contribution and test the significance of these two effects, we ran three parallel

ANOVAs, on FDp (i.e species turnover and intra-specific effects), on FDf (i.e. species turnover effect) and on their differences (i.e. intra-specific trait variability effect). The total sum of squared (SS) of each ANOVA was decomposed into the variability explained by each individual term according to altitude and an error term (i.e. unexplained variability). We defined a covariation effect on total variability to consider the positive (i.e positive covariation term) or negative (i.e negative covariation term) correlation between species turnover and intra-specific effects [36].

Statistical analyses were carried out using the software R 3.1.0 [38] and the packages *ade4*, *nlme, FD and gplots* and *trait.flex.anova* function [22] to decompose effect of intra-specific trait variation and/or species turnover on FD response to altitude.

RESULTS

The principal components analysis (Figure 2) identified general patterns of variation as well as relationships between functional traits and species. The first axis was associated with SLA, δ13C and individual size, explaining approximately 40% of total variance, whereas the second axis was related to N and height, explaining about 20% of total variance. Most species were ordered along the first axis with *F. curvifolia* and *J. communis* subsp. *alpina* and were associated with high values of individual size and δ13C (Figure 2, Table S1).

The correspondence between plant trait values per species and altitude was weak for most traits. However, some species decreased in size with altitude, and others thickened their leaves (see, Table S2a, for more details). In the models where other environmental predictors were used, we found few but idiosyncratic responses (results not shown). For example, high levels of soil N were positively linked to δ15N isotopes in the leaves of *P. valhii* (estimate = 0.54, p = 0.01), *J. humilis* (estimate = 0.44, p = 0.04), *F. curvifolia* (estimate = 0.49, p = 0.04) and *D. flexuosa* (estimate = 0.43, p = 0.01). Values of δ13C were negatively correlated to soil organic carbon in *A. caespitosa* (estimate = -0.17, p = 0.01) and *F. curvifolia* (estimate = -0.18, p = 0.03). Furthermore, the effect of altitude on the coefficients of variation for each trait and species was almost negligible in most cases (Table S2b).

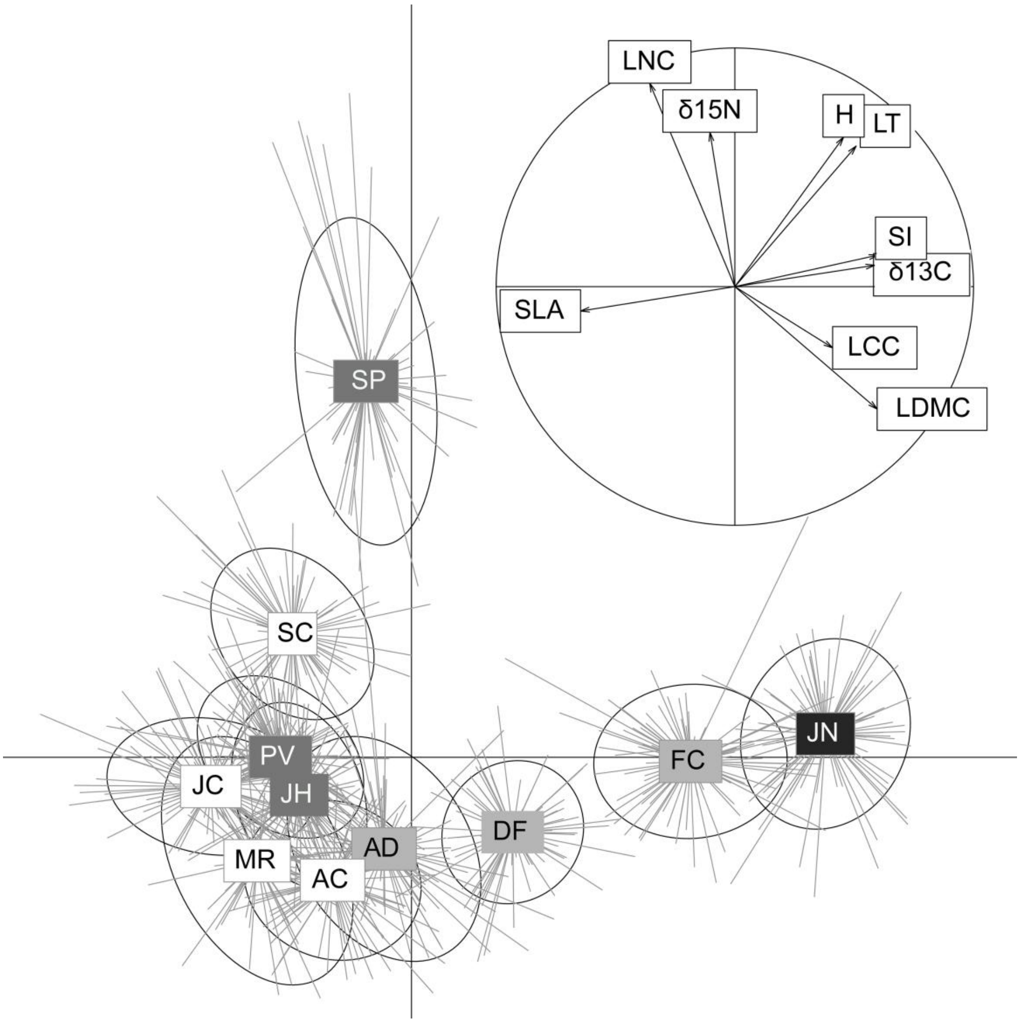


Fig. 2. Principal component analysis (PCAs) using the nine traits measured in eleven species for a total of 880 individuals. The variability of individuals of each species and their distributions along trait axes is represented in the main PCA by lines that arise from species mean value and by an ellipse of dispersion. The minor PCA shows the correlation between the nine traits using all data. Species names are included by using acronyms and colour-coded by their growth form: (i) Hemicryptophyte (dark grey): PV (*Pilosella vahlii*), SP (*Senecio pyrenaicus*), JH (*Jurinea humilis*); (ii) cushion chamaephyte (white): AC (*Armeria caespitosa*), JC (*Jasione crispa* subsp. *crispa*), MR (*Minuartia recurva*), SC (*Silene ciliata*); (iii) caespitosous hemicryptophyte (light grey) FC (*Festuca curvifolia*), DF (*Deschampsia flexuosa*), AD (*Agrostis delicatula*); (iv) shrub JN (*Juniperus communis* subsp. *alpina*). Acronyms for traits: plant size (IS), plant height (H), leaf thickness (LT), specific leaf area (SLA), leaf dry matter content (LDMC), leaf carbon content (LCC), leaf nitrogen content (LNC), carbon and nitrogen isotopes ratios (δ13C and δ15N, respectively).

Patterns of variance partitioning within and between sites were similar for most species and plant functional traits, although slight differences can be observed (Figure 3). In general, greater variation was found for all species within sites than between sites (on average 65% of the variance corresponded to within-site variation, whereas 35% corresponded to between-site variation).

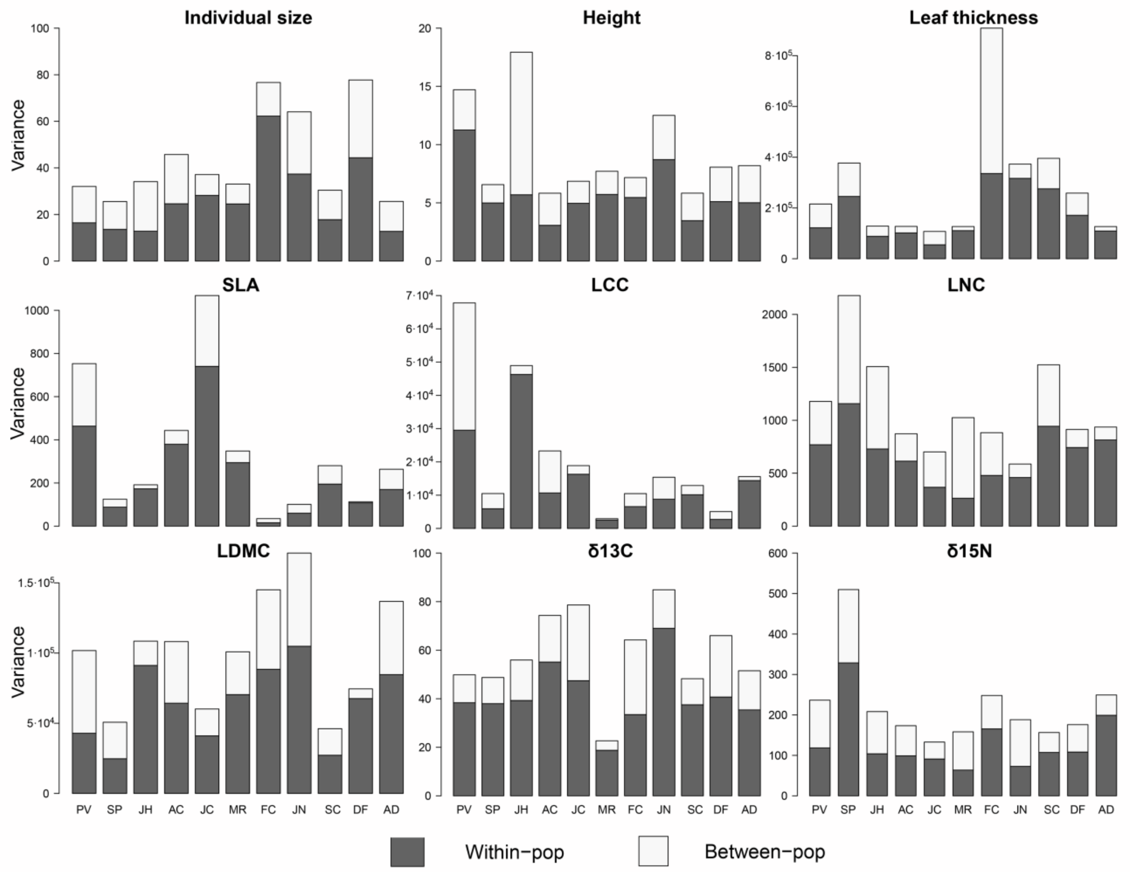


Fig. 3. Partition of variance between and within sites for each species and trait. Decomposition of variance into two levels, within and between sites (grey and white bars, respectively), obtained from an analysis of variance model for each of the eleven species and nine traits. Species and trait labels are the same as in Figure 2.

Community weighted means were quite similar, regardless of whether we regard the mean trait value at each site (CWMp) or across all sites (CWMf; paired t-test p-value > 0.68 in all cases). No significant changes in CWMf or CWMp were detected along altitude for any of the functional traits. The inter-specific FD was generally greater than that of the intra-specific component (Figure 4). In some cases, the two components were similar (e.g. LCC), while in others, intra-specific variability was significantly higher, as in δ15N and LNC, or δ13C. Total and inter-specific FD increased significantly with altitude in the case of SLA ($F_{1-7} = 31.92$; $p < 0.01$), LDMC ($F_{1-7} = 15.45$, $p < 0.01$), δ13C ($F_{1-7} = 19.33$, $p < 0.01$) and δ15N (only for inter-specific FD; $F_{1-7} = 8.8$, $p = 0.02$). When we separated the effect of species turnover and/or intra-specific trait variability on FD response to altitude, we found a significant species turnover effect in the case of SLA (relative contribution of turnover = 65%, $p < 0.01$). Furthermore, the effect of intra-specific trait variability on FD response to altitude was relevant in δ13C (relative contribution of intra-specific variability = 18%, $p = 0.01$) and in δ15N (relative contribution of intra-specific variability = 29.8%, $p = 0.01$). See table S3 for more details.

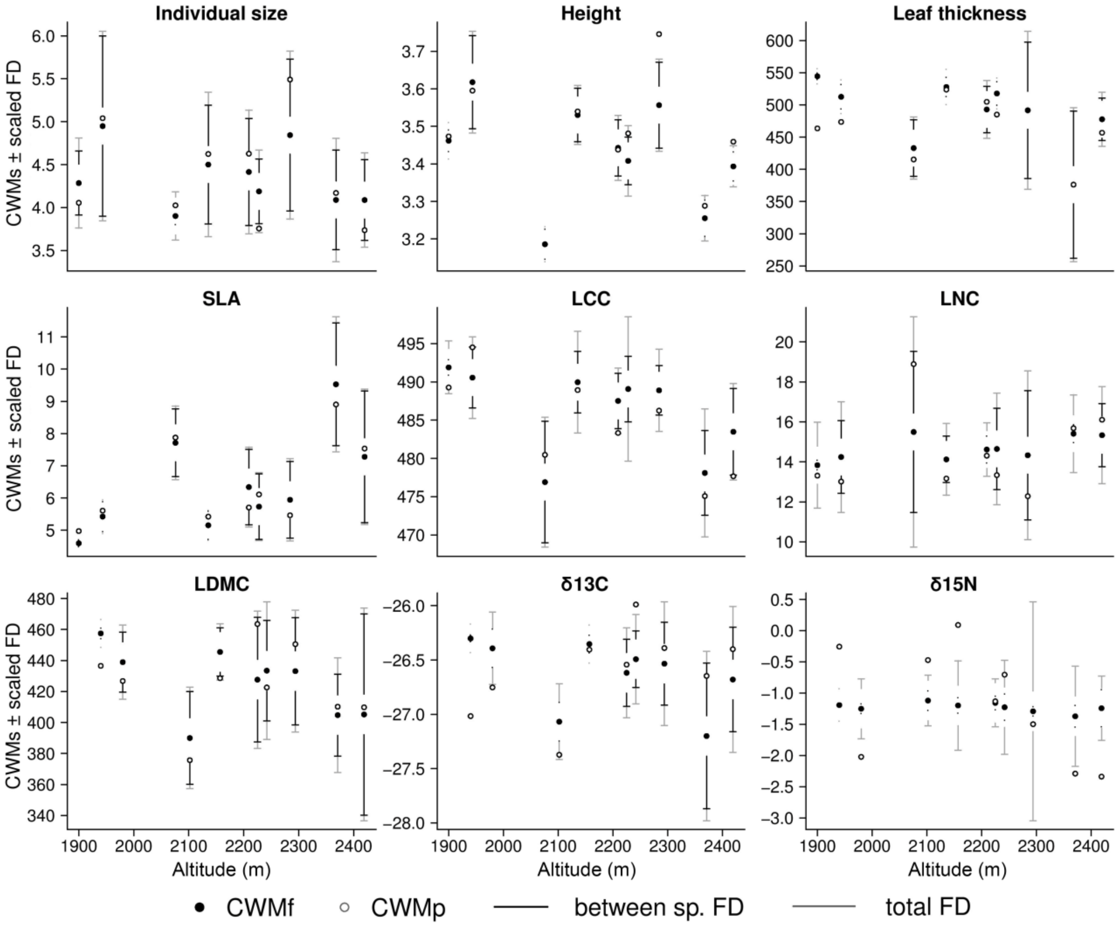


Fig. 4. Variation of community weighted means values (CWMs) and scaled functional diversity (FD) along altitudinal gradient for each trait. Partition of scaled FD into inter- and intra-specific variability (difference between Total – inter-specific FD) expressed as vertical bars around two CWMs values: using mean trait value measured for each species across all sites (CWMf – black dots) and mean trait value at each site (CWMp – white dots). Trait labels are the same as in Figure 2.

DISCUSSION

Our study highlights the importance of decomposing trait variability at different levels when assessing plant trait response to abiotic factors even in communities with low species turnover. Surprisingly, within-site trait variability at the individual species level was greater than between-site trait variability. Altitude had a weak effect on this variation, even though the species were located along a large gradient. At the community level, inter-specific FD component was generally more important than intra-specific component, nevertheless the effect of this on FD response to altitude was evident in some traits.

Individual species responses along gradients

Although specific trait shifts along environmental gradients have been described as a general pattern, we did not detect significant changes in mean trait values along the altitudinal gradient for most species. Nevertheless, a trend of decreasing individual size and increasing leaf thickness along altitude was observed in some species. This trend was also observed along some edaphic gradients in the case of δ13C and δ15N.

Decreasing individual size with altitude is concordant with the growth-limitation hypothesis [39]. In tree and shrub life-forms, low temperatures limit the formation of new cells and tissues, increasing the store of non-structural carbohydrate compounds mainly in root tissues. This effect was observed in *Juniperus* sp. [40], but to our knowledge, it has not been observed in alpine herbs or creeping chamaephytes. Increasing leaf thickness with elevation has also been described [41]. Recent studies have related this increase to UV-B radiation and the need to protect underlining photosynthetic systems [42]. Complementary mechanisms may inhibit cell expansion in drier environments [43], which would be compatible in our system where drought is more pronounced at low altitudes (Figure S1). Furthermore, some authors [44] have suggested a strong link between plant δ15N and N of soil in alpine ecosystems. This would explain our findings of a significant positive relationship between δ15N values and levels of soil N for *P. vahlii*, *J. humilis*, *F. curvifolia* and *D. flexuosa*.

Although we postulated a similar pattern among the species in our community in response to altitudinal gradient, our findings were far from clear. The long-term response of each species depends on its biogeography and selective forces, while species response at smaller spatial-temporal scales depended on its ability to face micro-environmental heterogeneity and/or biotic interactions. There is usually not a sole conclusion in the study of these patterns [9, 45], as species can have multiple or even opposite responses to one or more environmental gradients. Thus different functional traits may respond to different factors (e.g. δ13C is related to aridity gradient [46] while SLA respond to temperature more intensely [47]). In our case, and denoting a striking difference with other mountain ecosystems where moisture availability is not a problem, we found two opposed severity gradients operating along altitude (drought, which increased at lower altitudes; and freezing cold, as altitude increased; Figure S1). These two forces could explain our results to a great extent, since net responses can become neutral.

Within- and between-site variance partitioning

Between-site variability was expected to be the main source of trait variability at the species level, because the strong habitat filtering would select the best trait combination at each site. However, the highest level of trait-variance for individual species occurred within sites. Greater values of within-site variability were also found in a study conducted in a very heterogeneous temperate mountain ecosystem [9]. These authors expected the opposite results, because they used very small fragments with high genetic similarity in homogeneous micro-environments. Genetic variability and phenotypic plasticity represent two factors that determine intra-specific variability in different environments [23, 48]. Although these two factors are not disentangled using our experimental design, parallel studies carried out in this mountain range revealed high within-site genetic variability in two of our study species (*S. ciliata* [49] and *A. caespitosa* [50]). This relatively high genetic variability could explain our results. Furthermore, soil heterogeneity and micro-topography at fine spatial scales have proven to be as good predictors of plant performance as altitude in alpine ecosystems [51]. Our results suggest that some species and traits could be affected by local heterogeneity rather than by strong environmental gradients within their realized niche.

Patterns at the community level

The great within-site variability obtained can help us to understand the low variability observed in the CWMs along altitude at the community level. Previous studies have found that CWMs showed consistent variation under different gradients [52, 53]. Such shifts in CWMs could be mainly related to composition changes along gradients, unlike our case where species composition remained similar (73% of species were found in 89% of the sites). Therefore, the low variation in trait values between sites and the lack of shifts in CWMs confirm that abiotic filters are operating in the community at larger scales [53].

The partition of total functional diversity showed the extent of intra-specific FD was lower than inter-specific FD for 5 out of 9 plant traits (20% intra-specific variability). Although intra-specific trait variability has recently gained strength in the theory of coexistence [54], most studies have found that inter-specific variation is as high and similar as in our study (review Table 1 in [10]). This is surprising, as we expected a higher prevalence of intra-specific variability due to small species turnover [23]. Among the traits with an exceptionally high proportion of intra-specific FD were those associated with nutritional content or some metabolic pathways (δ15N and leaf N with 76% and 45% intra-specific FD, respectively), as reported in other studies [10, 16, 45]. These results could be at least partially due to the great effect of soil nutrient availability (which is very variable at small spatial scales in stressful ecosystems) on these traits and/or to genetic differences within species [10, 29].

The relationship between total and/or inter-specific FD and altitude showed a significant shift in 4 out of 9 plant traits. Namely, the highest sites were functionally more diverse for SLA, LDMC, δ13C and δ15N (only for inter-specific FD). An analogous study conducted in the Alps detected the opposite pattern for SLA and LDMC, finding greater diversity in these traits at low elevations [53]. In contrast to temperate mountains such as the Alps, Mediterranean high mountains are characterized by the presence of two opposing gradients: cold temperature *versus* summer drought. Water limitations at low altitudes (Figure S1) could cause greater environmental filtering and more abiotic stress [55, 56], which would result in inter-specific trait convergence [57, 58]. Under this scenario, conditions on summits are more favourable as soil water content is higher; so species must maximize their trait dissimilarity with different strategies to coexist and overcome resource competition [52].

On the other hand, the role of intra-specific variability is evident in the study of patterns along different gradients and has helped us to understand the response of FD to environment [19]. For instance, the relative contribution of intra-specific trait variability on FD response to altitude was significant for δ13C and δ15N (table S3). Although we selected a community with a smaller turnover than in most community ecology studies (β-diversity = 1.15), we found evidence of this pattern on FD response to altitude for SLA trait. This suggests that turnover can mask the real drivers and mechanisms governing community assembly within communities, which should be taken into account in future community studies based on traits. Furthermore, the low species turnover effect in our high mountain grassland, which might explain the lack of shifts in CWMs, provides a good opportunity to study the extent of intra- and inter-specific FD without strong compositional changes. Our study reveals the importance of considering both inter-specific and intra-specific trait variability in the evaluation of community functional trait structure along severity gradients. Although the FD pattern can be interpreted as a consequence of a strong abiotic filter when environment conditions become harsher, most of our species showed a weak functional response along altitude with higher within-site variability in most traits and species. Therefore, we recommend that further studies on functional diversity be carried out within communities to take measurements from more individuals of each species within each site.

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SUPPLEMENTARY MATERIAL

Table S1. Functional traits and species characterization. Means and SD (standard deviation) values of the nine functional traits of each of the eleven species sampled. The following species characteristics are shown: label, family, growth form (**HM cae**. - caespitososus hemicryptophyte; **Cs** -cushion chamaephyte; **Sh** - shrub; **Hm forb** – hemicryptophyte) and number of the sites where the species was sampled. Acronyms for the traits: leaf thickness (LT), Specific leaf area (SLA), leaf dry matter content (LDMC), leaf carbon content (LCC), leaf nitrogen content (LNC), carbon and nitrogen isotopes ratios (δ13C and δ15N respectably).

Species	Label	Family	Growth form	Sites	Individual size (cm^2)		Height (mm)		LT (µm)		SLA (mm^2·mg^{-1})		LDMC (mg·g^{-1})		LCC (mg·g^{-1})		δ13C (‰)		LNC (mg·g^{-1})		δ15N (‰)	
					mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Agrostis delicatula	AD	Poaceae	Hm cae.	7	23.45	14.96	20.48	6.79	209.1	42.9	11.43	1.95	397.3	44.5	449.5	15	-28.06	0.86	13.67	3.68	-0.96	1.90
Armeria caespitosa	AC	Plumbaginaceae	Cs	9	9.48	6.66	16.19	4.31	232.3	37.8	15.07	2.23	350.7	34.9	499.6	16.2	-29.38	0.91	14.18	3.13	-1.09	1.40
Deschampsia flexuosa	DF	Poaceae	Hm cae.	8	149.35	200.81	27.57	9.39	302.2	57.2	7.70	1.20	396.9	30.7	459	8	-27.30	0.91	12.04	3.40	-1.67	1.49
Festuca curvifolia	FC	Poaceae	Hm cae.	9	93.74	96.93	32.40	9.75	559.3	101	4.29	0.63	463.5	40.4	492.7	10.9	-26.24	0.85	13.76	3.15	-1.15	1.67
Jasione crispa subsp. *crispa*	JC	Campanulaceae	Cs	8	24.80	18.78	14.99	4.48	188	36.9	18.36	3.68	241.7	27.6	456	15.5	-28.86	1.00	19.76	2.98	-1.20	1.30
Juniperus communis subsp. *alpine*	JN	Cupressaceae	Sh	8	$1.1\cdot10^5$	$1.36\cdot10^5$	305.05	491.20	417.9	68.7	6.47	1.13	434.4	46.6	502.3	14	-25.70	1.04	12.25	2.73	-2.04	1.54
Jurinea humilis	JH	Asteraceae	Hm forb	9	28.66	18.45	6.96	3.31	262.3	38	10.11	1.47	266.3	34.9	478.5	23.5	-29.26	0.79	22.36	4.11	-1.52	1.53
Minuartia recurva	MR	Caryophyllaceae	Cs	6	31.27	28.44	16.71	6.95	111.2	53.1	17.16	2.78	340.2	47.4	454.1	8.1	-28.67	0.71	17.76	4.77	-1.56	1.87
Pilosella vahlii	PV	Asteraceae	Hm forb	9	15.43	9.62	21.98	8.88	278.1	49.2	16.73	2.91	207.7	33.8	459.5	27.6	-27.55	0.75	20.41	3.64	-2.54	1.63
Senecio pyrenaicus	SP	Asteraceae	Hm forb	6	35.86	32.01	313.85	89.59	486.7	79.9	10.29	1.45	189.6	29.3	453.9	13.3	-28.86	0.91	35.26	6.08	0.66	2.94
Silene ciliata	SC	Caryophyllaceae	Cs	8	38.81	26.81	19.58	5.13	353.1	70.8	14.06	1.88	207	24.2	440.9	12.8	-27.73	0.78	22.71	4.39	0.30	1.41

Table S2. Response of individual functional traits along altitude. A) Summary of linear mixed-effect models scores for each individual plant trait value and species, considering site as random factor and altitude as fixed factor; and B) lineal model scores with the coefficient of variation of each plant trait and species as the independent variable and altitude as fixed factor. Significant values ($p < 0.05$) are marked in bold.

A)

Lme (Ind. trait value~Altitud)	Individual size (cm^2)	Height (mm)	LT (µm)	SLA (mm^2·mg^{-1})	LDMC (mg·g^{-1})	LCC (mg·g^{-1})	δ13C (‰)	LNC (mg·g^{-1})	δ15N (‰)
	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate
Agrostis delicatula	**$-3.06\cdot10^{-4}$**	$-1.00\cdot10^{-4}$	$-7.90\cdot10^{-3}$	$5.30\cdot10^{-4}$	0.078	$-1.24\cdot10^{-3}$	$-1.25\cdot10^{-3}$	$2.51\cdot10^{-4}$	$-3.36\cdot10^{-3}$
Armeria caespitosa	$-2.32\cdot10^{-5}$	$8.53\cdot10^{-5}$	$-8.19\cdot10^{-3}$	$1.84\cdot10^{-3}$	-0.058	$3.04\cdot10^{-3}$	$1.57\cdot10^{-3}$	$1.43\cdot10^{-4}$	$-2.41\cdot10^{-3}$
Deschampsia flexuosa	$-7.60\cdot10^{-5}$	$1.81\cdot10^{-4}$	**0.167**	$-1.35\cdot10^{-3}$	$2.87\cdot10^{-3}$	$1.98\cdot10^{-3}$	**$-2.40\cdot10^{-3}$**	$4.99\cdot10^{-4}$	$-3.59\cdot10^{-3}$
Festuca curvifolia	$1.82\cdot10^{-5}$	$7.82\cdot10^{-5}$	0.215	$-1.58\cdot10^{-3}$	0.097	$-1.42\cdot10^{-3}$	**$3.33\cdot10^{-3}$**	$1.40\cdot10^{-4}$	$-5.74\cdot10^{-3}$
Jasione crispa subsp. *crispa*	$-1.77\cdot10^{-4}$	$-2.98\cdot10^{-5}$	-0.014	$2.02\cdot10^{-4}$	0.017	$8.16\cdot10^{-4}$	$1.74\cdot10^{-3}$	$-7.26\cdot10^{-4}$	$6.56\cdot10^{-4}$
Juniperus communis subsp. *alpina*	**$-2.59\cdot10^{-3}$**	$-1.99\cdot10^{-4}$	-0.0149	$2.19\cdot10^{-3}$	-0.07	$-3.11\cdot10^{-3}$	$2.56\cdot10^{-4}$	$1.06\cdot10^{-4}$	$-4.22\cdot10^{-3}$
Jurinea humilis	$-1.01\cdot10^{-4}$	$1.16\cdot10^{-4}$	**0.1**	$-9.90\cdot10^{-4}$	0.019	$1.69\cdot10^{-3}$	**$-2.31\cdot10^{-3}$**	$7.19\cdot10^{-4}$	$-1.92\cdot10^{-3}$
Minuartia recurva	$1.98\cdot10^{-4}$	$4.66\cdot10^{-4}$	-0.121	$-7.72\cdot10^{-3}$	**0.213**	$5.88\cdot10^{-4}$	$-8.00\cdot10^{-6}$	$-3.07\cdot10^{-3}$	-0.011
Pilosella vahlii	$-2.16\cdot10^{-4}$	$1.06\cdot10^{-4}$	**0.172**	**$8.34\cdot10^{-3}$**	**-0.137**	$1.54\cdot10^{-3}$	$3.61\cdot10^{-5}$	$-1.59\cdot10^{-4}$	**$-5.77\cdot10^{-3}$**
Senecio pyrenaicus	**$-3.15\cdot10^{-4}$**	$3.22\cdot10^{-5}$	0.196	$1.57\cdot10^{-3}$	-0.059	$-2.29\cdot10^{-3}$	$-1.15\cdot10^{-3}$	$-1.97\cdot10^{-4}$	$3.89\cdot10^{-3}$
Silene ciliata	**$-3.13\cdot10^{-4}$**	$-1.48\cdot10^{-4}$	**0.225**	$-2.80\cdot10^{-3}$	0.024	$9.73\cdot10^{-4}$	$1.37\cdot10^{-3}$	$-1.04\cdot10^{-3}$	$8.51\cdot10^{-5}$

Table S2. Cont.

B)

Lm (CV trait value~Altitud)	Individual size (cm^2)	Height (mm)	LT (µm)	SLA ($mm^2\cdot mg^{-1}$)	LDMC ($mg\cdot g^{-1}$)	LCC ($mg\cdot g^{-1}$)	δ13C (‰)	LNC ($mg\cdot g^{-1}$)	δ15N (‰)
	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate	Estimate
Agrostis delicatula	$-4.94\cdot10^{-3}$	$2.79\cdot10^{-3}$	$8.97\cdot10^{-3}$	**$8.42\cdot10^{-3}$**	**0.02**	-0.088	-0.030	$4.43\cdot10^{-3}$	$-4.09\cdot10^{-3}$
Armeria caespitosa	$3.68\cdot10^{-3}$	$-7.72\cdot10^{-3}$	$1.26\cdot10^{-3}$	$4.11\cdot10^{-4}$	$-6.60\cdot10^{-3}$	0.043	0.027	$3.59\cdot10^{-3}$	$-1.86\cdot10^{-3}$
Deschampsia flexuosa	$2.19\cdot10^{-3}$	-0.011	$1.82\cdot10^{-3}$	$-6.02\cdot10^{-4}$	$3.33\cdot10^{-4}$	$1.19\cdot10^{-3}$	$-2.08\cdot10^{-3}$	$-4.30\cdot10^{-3}$	$-1.63\cdot10^{-3}$
Festuca curvifolia	$-1.48\cdot10^{-3}$	$-2.66\cdot10^{-3}$	$-3.21\cdot10^{-3}$	$1.00\cdot10^{-3}$	$-1.93\cdot10^{-3}$	0.051	0.019	$2.42\cdot10^{-3}$	$-2.59\cdot10^{-3}$
Jasione crispa subsp. *crispa*	**$7.05\cdot10^{-3}$**	$8.26\cdot10^{-3}$	$-6.25\cdot10^{-4}$	0.015	0.017	-0.090	$-2.06\cdot10^{-3}$	0.01	$-1.87\cdot10^{-3}$
Juniperus communis subsp. *alpina*	0.011	$-9.32\cdot10^{-3}$	$4.97\cdot10^{-3}$	0.03	0.016	0.059	-0.015	$4.13\cdot10^{-3}$	$-7.38\cdot10^{-3}$
Jurinea humilis	$4.08\cdot10^{-3}$	-0.012	-0.011	$2.89\cdot10^{-3}$	$1.16\cdot10^{-3}$	-0.020	-0.013	$6.25\cdot10^{-3}$	$-2.90\cdot10^{-3}$
Minuartia recurva	0.011	$-7.79\cdot10^{-3}$	$1.51\cdot10^{-3}$	$8.59\cdot10^{-3}$	0.027	0.062	-0.055	**-0.012**	$-8.53\cdot10^{-3}$
Pilosella vahlii	$3.19\cdot10^{-3}$	0.014	-0.011	$6.64\cdot10^{-3}$	$4.27\cdot10^{-3}$	$-4.90\cdot10^{-3}$	0.020	$8.20\cdot10^{-3}$	**$-9.36\cdot10^{-3}$**
Senecio pyrenaicus	$-2.20\cdot10^{-3}$	0.027	0.012	$9.89\cdot10^{-3}$	$-5.06\cdot10^{-3}$	0.033	-0.018	$5.77\cdot10^{-3}$	$1.98\cdot10^{-3}$
Silene ciliata	$1.70\cdot10^{-3}$	-0.028	$-1.01\cdot10^{-3}$	$7.16\cdot10^{-3}$	**0.028**	0.091	-0.012	$2.17\cdot10^{-3}$	$5.44\cdot10^{-5}$

Table S3. Species turnover and intra-specific trait variability relative contribution (in %) on FD response to altitude. Using three ANOVAs and Sum of squared (SS) decomposition for FDp (species turnover and intra-specific effects), FDf (species turnover effect) and their differences (intra-specific trait variability effect), it is possible to establish the relative effect on FD response to altitude of species turnover and intra-specific trait variability for each functional trait considered. Covariation effect consider the positive (positive covariation term) or negative (negative covariation term) correlation between species turnover and intra-specific effects. Significant values ($p < 0.05$) are marked in bold.

Functional trait	Turnover effect	Intra-specific effect	Covariation effect	Total effect
Individual size (cm^2)	5.9	3.6	-9.3	0.3
Height (mm)	6.0	9.6	-15.2	0.4
LT (µm)	25.5	2.1	14.7	42.2
SLA ($mm^2 \cdot mg^{-1}$)	**65.1**	1.1	17.1	**83.4**
LDMC ($mg \cdot g^{-1}$)	27.7	7.5	28.8	**63.9**
LCC ($mg \cdot g^{-1}$)	62.9	10.4	-51.1	22.1
δ13C (‰)	19.9	**18.0**	37.9	**75.8**
LNC ($mg \cdot g^{-1}$)	7.6	3.7	-10.6	0.7
δ15N (‰)	4.6	**29.8**	23.5	**57.9**

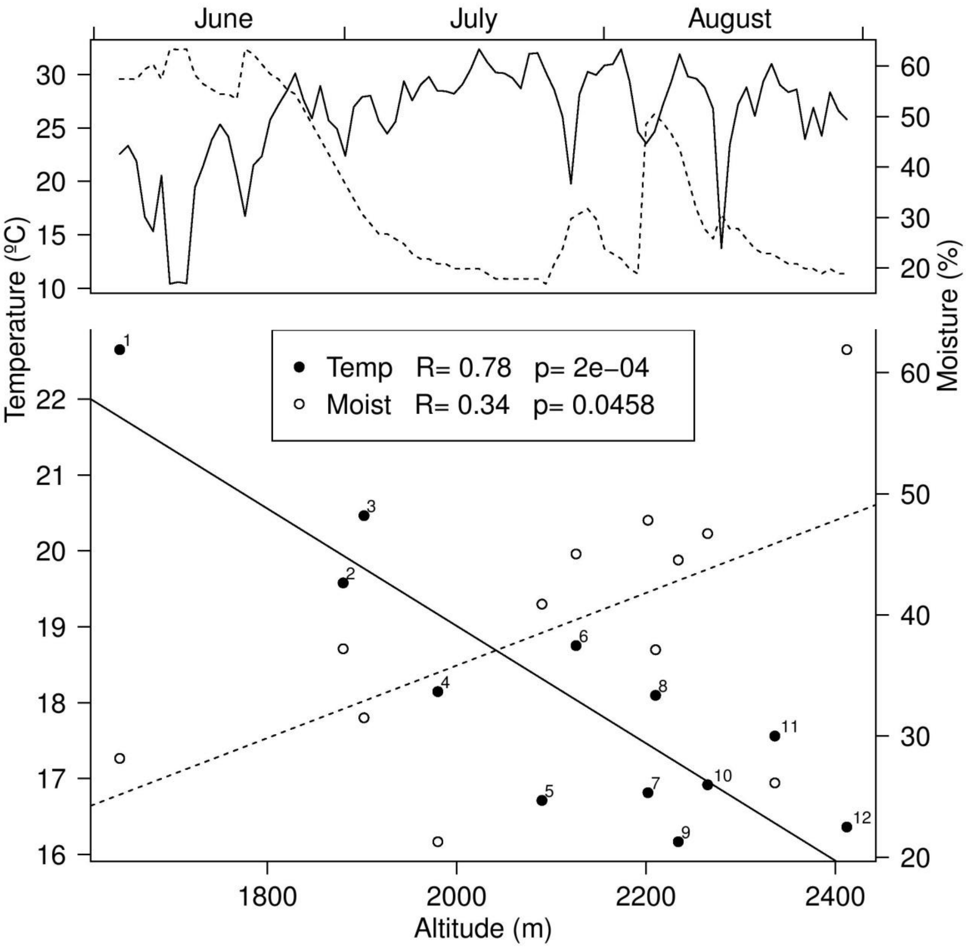

Figure S1. Soil temperature and moisture in the study area. Changes in soil Temperature (ºC) and Moisture (%) during a growing season (June-August), considering mean region values (upper panel) and values at different sites along the altitudinal gradient in the study region (lower panel). These climatic variables were estimated at the following sites: 1 (Porrones, X = 420744, Y = 4512188); 2 (Collado de las Vacas, X = 419169, Y = 4513361); 3 (Loma Cabezas, X = 419960, Y = 4514311); **4 (Tirobarra, X = 408746, Y = 4518015)**; **5 (Najarra, X = 430197, Y = 451880)**; 6 (Nevero M, X = 428802, Y = 4537552); 7 (Nevero P, X = 428759, Y = 4537426); **8 (Bola, X = 417587, Y = 4515896)**; 9 (H. Menor, X = 418456, Y = 4521166); **10 (H. Mayor, X = 418843, Y = 4522259)**; **11 (C. H. Mayor, X = 421276, Y = 4516840)**; **12 (Peñalara, X = 419443, Y = 4522832).** Bold names denote sites where traits were also measured.

III

Local heterogeneity balancing environmental filtering between sites in a Mediterranean high mountain community

Pescador, D., de Bello, F., Valladares, F. and Escudero, A.

Manuscrito inédito

ABSTRACT

Niche theory has explained the non-random assembly patterns through environmental filtering and niche differentiation focusing on functional and phylogenetic distances. Despite progress in evaluating the plant coexistence, the knowledge about how the scale plays an important role in underlying assembly processes remains unknown. Here, we assessed the functional and phylogenetic variance sources and the patterns of convergence and divergence considering different spatial scales in a high mountain community located in Sierra de Guadarrama (Spain). The variation in community weighted mean trait value (CV-CWMs), variance partition within/between plot of mean pairwise distance and divergence/convergence patterns were evaluated in three nested scales (39 plots of 20 x 20 m, divided in 195 subplots of 2.4 x 2.4 m and 2496 cells of 0.3 x 0.3 m) using 5 functional traits and the phylogenetic tree for 56 species. The CV-CWMs at cell scales was in most cases similar to the variation in this source at global scale. The variance partition showed most variance within plots for all resources of diversity. Functional divergence was more patent at inter-medium scales (i.e. subplots). These results suggest that niche differentiation modules the species coexistence at finest scales through micro-heterogeneity or species interactions. Finally at the largest scale functional convergence was the dominant pattern (i.e. coexisting species are more similar), which suggest environmental filtering. These findings showed the existence of a primary filter which defines a same regional pool across all community (i.e. low turnover). The species coexistence under this filter is produced according to assembly rules where the niche differentiation at fine scale represents the genuinely important pattern.

INTRODUCTION

Paradigms behind community assembly are based on the role played in the community by coexisting species, which is conditioned by a set of abiotic, biotic or historical drivers. These include all processes favouring or obstructing the presence of species in a particular assembly at a given spatial and time scale. Approaches based on ecological distances among co-occurring species using plant functional traits or phylogenetic relatedness have been recently proposed and profusely used as better predictors than species taxonomic diversity to investigate underlying processes and provided ecosystem services (Cadotte *et al.*, 2011; Helmus & Ives, 2012).

Plant functional traits represent any measurable morphological, physiologic and ecological characteristic that potentially affects fitness, performance and in summary the ability to establish and survive under certain environment conditions (Violle *et al.*, 2007). They are powerful indicators of plant strategies to face biotic and abiotic constraints in the constitution of realized communities and therefore can be used in order to explain what are the drivers governing community assembly. In addition the development of phylogenetic community ecology, which assess the evolutionary distances between co-existing species within a community, may help to understand how communities are assembled by explicitly considering the evolutionary history of the local pool of species (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009; Kraft & Ackerly, 2010; Mouquet *et al.*, 2012). This idea has been traditionally based on a very simply and in some reductionist idea: closely related species are likely more functionally similar than lineages most distant. Nevertheless recent studies has showed that the phylogeny plays a relatively small role when community assembly processes are studied at fine scales (Swenson & Enquist, 2009; Liu *et al.*, 2013).

Niche-based theory has identified two major forces that configure the realized assemblages: environmental filtering and limiting similarity. The concept of environmental filtering implies that the community are configured by species that through a suitable functional traits suite have surpassed the set of hierarchical filters imposed by the physical environment. As a consequence if this mechanism is ruling out species found are expected to show similar requirements and thus the community is more likely typified by trait convergence (Weiher & Keddy, 1995; Leibold, 1998). Environmental filtering is predicted in areas with a marked abiotic factor that limit plant productivity, whose influence extends from regional (e.g. temperature) to local (e.g. micro-topography) scales. In the other hand, limiting similarity (*sensu* MacArthur & Levins, 1967) predicts that the competence between species with similar requirements produce a niche segregation and a community with coexisting species more different than expected by chance (Mason & Wilson, 2006). This would imply trait divergence for exploiting such diversity of niches. Nevertheless trait convergence may be also linked to biotic processes when exist a dominance of closely related species with a strong competition ability that excludes weakest competitors (Chesson, 2000; Mayfield & Levine, 2010; de Bello *et al.*, 2012). As a rule, competitive interactions between individuals from different species occur at finer spatial scales and therefore convergence or divergence caused by biotic effects will arise at this scale (e.g. (Kraft & Ackerly, 2010). Alternatively, co-existence among species may be a consequence of stochastic dynamics and dispersal limitation if all species are ecologically similar (Neutral perspective; (Hubbell, 2001).

Despite the spatial scale dependence of these processes, traditionally their importance has been studied at a single spatial scale, but see (Kraft & Ackerly, 2010;

Messier *et al.*, 2010; de Bello *et al.*, 2013). This is surprising since it is well known that the "community" and the "species pool" are spatially defined at any scale although the interpretation of resultant patterns varies across spatial scale. To describe the functional and phylogenetic community structure a multi-component approach has been developed based on several axis of diversity (i.e. functional – FD – and phylogenetic – PD – diversity respectively), which may be quantified as regional, local and among-sites components (i.e. gamma, alpha and beta diversity respectively in the more widely interpretation of diversity components). Approaches based on alpha diversity have been used to identify the mechanisms at local-scale. Investigations into beta diversity, which often is defined as the change in species composition or turnover between two or more species assemblages (Whittaker, 1960; Whittaker, 1972; Cody, 1975) are more recent (Anderson *et al.*, 2011). It represents a link among alpha and regional diversity and is critical in the search of underlying assembly mechanisms. The functional component may be completed using the dominant trait values at a particular scale, often developed as the community weighted mean (CWM; (Garnier *et al.*, 2007; Violle *et al.*, 2007)). If this component is considered repeatedly across a same scale, a measure of functional heterogeneity may be defined, which presumably increases with scale as the environmental filter constrains less species.

In this study we analyze functional traits and phylogenetic information collected in a Mediterranean high mountain community along an altitudinal gradient by following a multi-scale approach to test the importance of random, abiotic and biotic processes for community assembly. First, we assessed the functional heterogeneity considered as variation in CWM values (CV-CWMs) at each scale and then we hypothesized a higher importance of this resource at broad scales in response to increased ecological niches.

We evaluated the convergence and divergence patterns on the FD and PD across scales using null model approach and studied the correlation versus several environmental variables. Thus we hypothesized that environmental filtering will act mainly at large-scale defining the assembly community characterized by a convergence pattern. While biotic effects will act at more fine-scale drive to divergence pattern. Alternatively biotic interactions may cause convergence when a closely relative competitors domain the assembly while habitat heterogeneity could theoretically lead to divergence. Therefore we studied the relationship among CV-CWMs and convergence-divergence patterns to provide signals of underlying assembly patterns. This community represents an ideal system to test these hypothesis, with a strong environmental filtering that excludes the species vulnerable to cold (Körner, 2003) and with a similar species pool across sites (i.e. low species turnover), which have to deal with several abiotic and biotic constrains.

MATERIALS AND METHODS

Study Site and field sampling

The study was conducted above the treeline at *Sierra de Guadarrama* National Park on the northwest of Madrid, Spain (40º46´39´´ to 40º51´8´´ N; 3º49´44´´ to 4º4´59´´W; 1940 m – 2419 m a.s.l.). The climate is Mediterranean, with a mean annual temperature and precipitation of 6.4ºC and 1350 mm respectively and with a drought from May to October (less than 10% of the annual precipitation), which is more evident at lower altitudes.

Here, the treeline is located between 1900 and 2000 m a.s.l. is dominated by Scots pines (*Pinus sylvestris* L.) interspersed in a shrubby matrix of *Cytisus oromediterraneus* Rivas Mart. *et al*. and *Juniperus communis* L. subsp. *alpina* (Suter) Čelak. Above this limit the dominance of shrubby matrix up to 2100-2200 m a.s.l with dry and cryophilic grassland in less pronounced locations. In the highest areas (from 2200 to 2419 m a.s.l.) this grassland represents the natural vegetation in zones on windblown slopes and summits. This plant community is organized in a mosaic of patches dominated by *Festuca curvifolia* Lag. ex Lange and other creeping and cushion chamaephytes clumped in a bare ground matrix.

In the summer of 2011 we selected 39 sites covering the whole altitudinal range of *Festuca curvifolia* grassland community and established a sampling plot of 20 x 20 m (1,56 ha). In each plot we sampled the vegetation at three different scales, cell, subplot and plot scale. First a 2.4 x 2.4 m square was randomly located and divided into 64 cells of 30 x 30 cm, which represent the cell scale (the finest scales). Second, five subplots of 2.4 x 2.4 m (including the grid of cells) were established as the intermediate scale. The cover of every plant species, soil and rock was visually and directly estimated at each cell and subplots. Finally the total cover at plot scale was indirectly estimated as the average of five subplots after having estimated the cover of additional plant species outside subplots.

Each plot was characterized by a set of environmental variables that may predict changes in species composition between plots. Specifically we measured the altitude and orientation using a GPS (Garmin Colorado-300) and the slope using a clinometer (Silva Clinomaster CM-360-%, LA). Orientation and slope values were employed to estimate the insolation coefficient following Gandullo´s method. Finally a total of fifteen soil samples (5 cm of diameter, 10 cm depth) were randomly collected in each plot in three different micro-habitats (five samples for habitat): bare ground, under vegetated patch dominated by *F.curvifolia* and under shrub influence. Soil samples were sieved (2 mm mesh) and air-dried for 1 month to estimate pH using a pH meter GLP 21 (Crison, Barcelona, Spain), soil organic carbon (hereafter SOC, in %) by colorimetry after oxidation with $K_2Cr_2O_2$ and H_2SO_4 and total N (mg N g^{-1} soil) on a SKALAR++ San Analyzer (Skalar, Breda, The Netherlands) after digestion in H_2SO_4 and Kjedahl´s catalyst.

Plant Functional Traits

During summer 2011 and 2012 multiple plant functional traits were measured in a total of 56 species in five of most diverse previous plots (See Table S1). These species represent more than 83% of species in the community and cover around the 99% (±1) of relative plant abundance. A minimum of 10 individuals per species were selected in at least one of the five sites and five functional traits were measured: plant height (Hmax;

length between the ground and the top of photosynthetic tissues), foliar thickness, specific leaf area (SLA; ratio between leaf fresh area and its dry weight), leaf dry matter content (LDMC; ratio between leaf dry weight and its fresh weight) and seed mass.

DNA isolation, Sequencing and Phylogenetic analysis

We collected fresh young leaf from three individuals per species that was dry-stored in silica-gel for one month. Up to 20 mg of dry-leaf sample was disrupted and homogenized with a TissueLyser LT (Quiagen, Valencia, CA) using glass beads. Total genomic DNA was extracted with DNeasy Plant Mini-Kit (QIAGEN, Valencia, CA), according the manufacturer's instructions. Standard polymerase chain reactions (PCRs) were used to amplification of primary barcoding loci *rbcLa* and *matK*. For amplification, 2-µL of DNA was added in 23-µL reaction [2.5 µL of Taq buffer 2 mM with$MgCl_2$, 1 µL of dNTP Mix (0.4 mM), 1.25 µL of reverse and forward primer and 1.25 U Taq DNA Polymerase (Biotools, Madrid, Spain)]. The PCR was performed in a S1000 Thermal Cycler (Bio-Rad, USA), using the different cycling program. The PCR products were clean-up using ExoSap-IT ® (USB Corporation, Cleveland, OH) and submitted to Macrogen Inc (Seoul, South Korea) for sequencing in forward and reverse directions. A total of 288 sequences (3 individuals per species·[56 species successful sequencing with rbcL + 40 species successful sequencing with matk]) were open with Sequencher 4.1.4 (Genes Code Corporation) and contigs of each species were assembled from forward and reverse secuences. *RbcL* and *MatK* markers were aligned independently using Mafft online-version 7 (Katoh & Standley, 2013) following the G-INS-I strategy, recommended for <200 sequences with global homology. Mac Clade 4.06 (Maddison & Maddison, 2003) was used to concatenate primer configurations

(*RbcL* + *MatK*) and created one supermatrix with missing data when the marker could not be sequenced.

We reconstructed the phylogeny of our community using Bayesian inference (BI). Previously we tested the best-fit models of nucleotide substitution in jModelTest 2 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) using Akaike Information Criterion (AIC). Bayesian analyses were performed with five parallel MCMC (Markov Chain Monte Carlo) in Mr Bayes 3.2 (Ronquist & Huelsenbeck, 2003) with the following parameters: 3 runs, 40 million generations and sampling a tree every 100 generations. Half of the number of trees constituted the burnin and final consensus tree was summarized from the remaining trees.

Metrics of community structure and statistical analyses

The community structure at different work scales can be assessed by different components. The component "community weighted mean" (CWM) represents the mean trait value of a community considering the trait value and the relative abundance of each species at a specific site (measured here as species cover). Here, we calculated the CWM value for each cell, subplot and plot and then we obtained a coefficient of variation value for each of 64 cells grid (CV-CWM between cells), 5 subplots set (CV-CWMs between subplots). Note that CV-CWM between cells may be considered as CV-CWM within subplots since the 64 cells grid area is equivalent to a subplot area. An only coefficient of variation was obtained for the 39 plots (CV-CWMs global). These components reflects the functional heterogeneity at cell, subplot or plot scale respectively and all of them were estimated considering all traits together (multi-trait approach) and for each of traits independently. The differences among each component were evaluated using a paired t-test.

Other component of community structure describes the diversity within a community. Similarly to CWM, functional diversity can be calculated at the different work scales and then decomposed in local, regional and among sites (so-called alpha, gamma and beta diversity respectively). In our study the beta component represents the diversity among cells, subplots or plots and so can be seen as a spatial functional turnover metric. This component in turn can be considered from a taxonomic, phylogenetic or functional perspective. With this in mind for each plot we calculated the beta diversity among the 64 cells or among 5 subplots using the inter-site mean pairwise distance (MPD) and considering three sources of diversity: taxonomic, phylogenetic and functional (multi-trait or single-trait approach). This index compares all possible pair of sites based on taxonomical, functional o phylogenetic distances of the species present in each site (measured here as Gower´s dissimilarity; Gower, 1971). Thus we assessed the differences among all sites according to species identity (Tax_{MPD}), phylogenetic distance (Phy_{MPD}) or functional distance (F_{MPD}). Inter-sites MPD matrices were used to explain how the variance for each resource of beta diversity was attributed to within site or between site though a permutational multivariate analysis or nonparametric manova (Anderson, 2001). Both the inter-site MPD among the 64 cells and among 5 subplots were used independently.

In order to assess the functional and phylogenetic divergence-convergence at different scales we calculated the mean pairwise distance (MPD_{obs}) between all individuals observed in each cell, subplot or plot. The distances between individuals were calculated based on Gower´s dissimilarity (Gower, 1971) using the plant functional traits and the cophenetic correlation for the phylogenetic tree. Each MPD_{obs} value was expressed relative to that MPD simulated (MPD_{exp}) from a null model to calculate a Standardized Effect Size (SES; Gotelli and McCabe, 2002) for each scale, trait, all trait together and phylogeny. To generate the null distribution we designed a realistic null model where a) the number of species and total cover of each cell, subplot or plot were remained fixed. b) The species names of each simulated assemblage came from the species names of plot to which the cell/subplot belonged (i.e. local pool) or from the species names of the whole plots (i.e. regional pool). So in case of cell and subplot scales the species that generate the null assemblage may be came to local or regional pool while for plot scale only the latest pool was regarded. c) The likelihood that one species appeared on null assemblage was directly linked to its abundance at plot or regional scale, as appropriate. d) The cover of each species in null distribution was proportional to its abundance at plot or regional scale. Based on these constraints we generate 1000 null assemblages for each cell (64 cells/plot * 39 plot), subplot (5 subplot/plot * 39 plots) and plot where MPD_{exp} values were estimated using each trait, all traits together and phylogeny. Comparing each MPD_{obs} to corresponding 1000 random MPD_{exp} values allowed us calculate SES of MPD as follows:

$$S.E.S.MPD = {}^{(MPD_{obs} - \overline{MPD_{exp}}}\Big/_{sd\ (MPD_{exp})}$$

where MPD_{obs} is the MPD obtained from the observed data at pertinent work scale and using the functional or phylogenetic distance, $\overline{MPDexp}$ the mean value of the 1000 null MPD distribution and $sd\ (MPD_{exp})$ the standard deviation value of the 1000 null MPD distribution. Standardized Effect Size values greater than 1.96 indicate a higher than expected dissimilarity between assemblage species (i.e. divergence) and values below -

1.96 indicate a lower than expected dissimilarity between assemblage species (i.e. convergence). In total, we calculated S.E.S MPD for 64*39 cells, 5*39 subplots and 39 plots considering each plant functional trait individually (i.e. Hmax, Thickness, SLA, LDMC, Seed mass), all traits together and phylogeny distances. In case of cell and subplot scale the null assemblage was built based on local or regional species pool consideration. Finally, the response of environmental predictors (altitude, insolation coefficient, soil pH, SOC and total N) on each S.E.S MPD was established by Pearson´s correlation index.

All statistical analysis were made with the software R 2.12.1 (R Core Team, 2012) and the following functions: dbFD and gowdis functions implemented in the FD package (Laliberté & Legendre, 2010; Laliberté & Shipley, 2011), adonis function of vegan (Oksanen *et al.*, 2013), mpd function of picante (Kembel *et al.*, 2010) and melodic function.

RESULTS AND DISCUSSION

Functional heterogeneity across scale

There was a similar trend of functional heterogeneity across scale (Fig. 1), with coefficient of variation of community weighted mean at global scale (CV-CWMg) generally greater than CV-CWM at subplot scale (CV-CWMb). In most cases this component was less than CV-CWM at cell scale (CV-CWMw), except to Seed mass where both components were similar. CV-CWMg and CV-CWMw were similar in case of SLA and LDMC trait (p-value paired t-test = 0.39 and 0.48 respectively). For multi-trait approach the paired t-test established a significant sorting as following: CV-CWMg > CV-CWMw > CV-CWMb.

These results suggest that in our system the environmental filtering between sites is not as strong as it might think. In high mountain communities the species composition is limited by a primary filter, such as the cold (Körner, 2003). It has been proposed that harsh conditions promote a similar species composition between sites (i.e. low turnover; Helmus and Ives, 2012). The heterogeneity at cell scale represents at least in some cases a source of variation similar to that observed at regional scale. This implies that micro-environmental gradients (Hutchings *et al.*, 2003; Burton *et al.*, 2011) or species interactions are promoting the coexistence at fine scales. More specifically, whether local heterogeneity modulates the community within a site the models that attempt to explore the community structure through the environmental condition and based on species functional traits (see Shipley et al., 2006) have their limits.

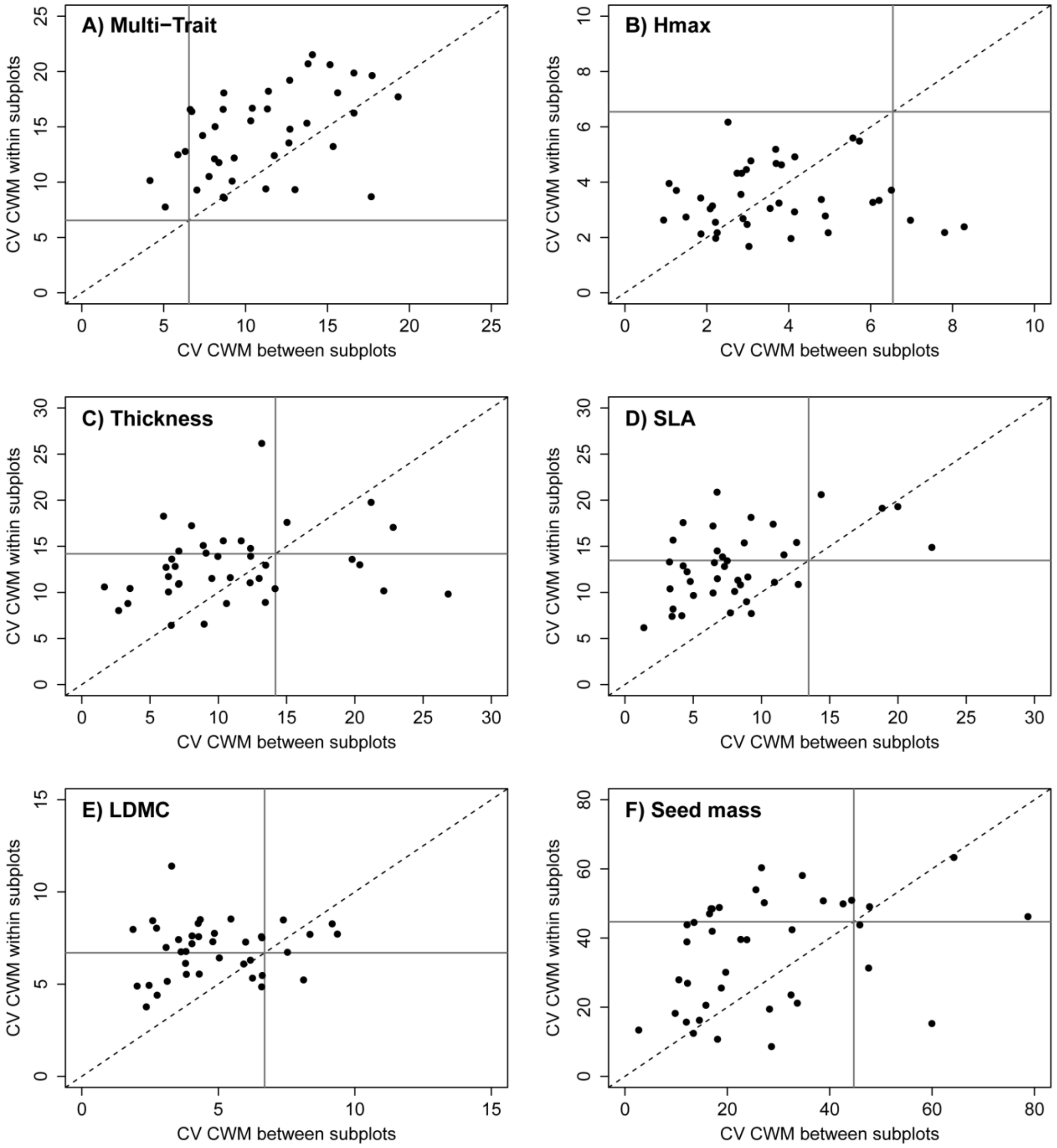

Fig. 1. Relationships between CV of CWMs at three work scales and for multi-trait approach and each single functional trait. CV of CWMs between subplots (CV-CWMb) are represented in x axis, CV of CWMs within subplots (CV-CWMw) in y axis and the global value of CV of CWMs are displayed with solid lines. Dashed line represents the relation 1:1.

Sources of Beta diversity

The partition of variance using mean pairwise distance (MPD) as a measure of beta diversity showed most of variance within plots for all resources of diversity (Fig. 2), regardless whether we used the 64x39 cell (around 86% versus 14% of variance mean) or 5x39 subplot data set (75% versus 25%). Taxonomic, phylogenetic and multifunctional beta diversity showed similar values of variance at both work scales. Changes in functional and phylogenetic distances between two cells or subplots were therefore proportional to changes in species identity between those cells or subplots. Single-trait consideration at cell scale was more variable, with beta diversity within plots near to 100 % in case of Seed mass (Fig. 2).

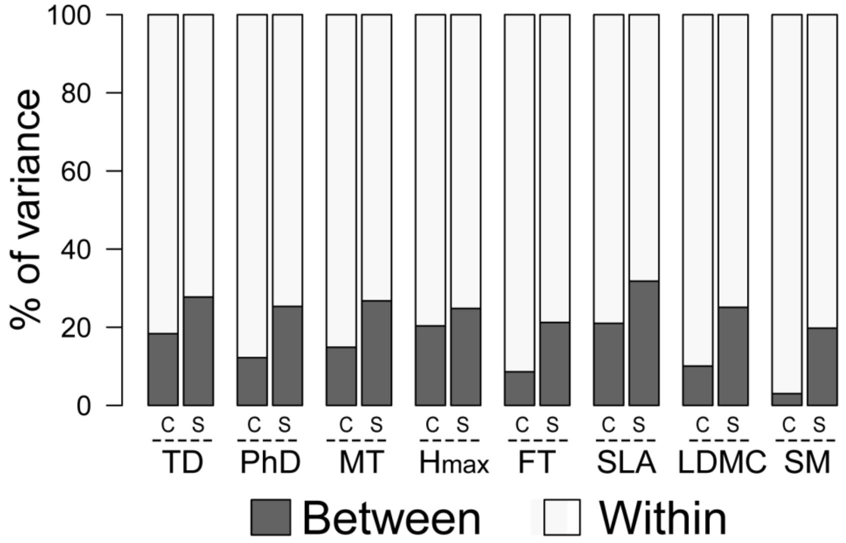

Fig. 2. Partition of variance between and within sites for each particular diversity resource. The analysis of variance was carried out fitting linear models and partitioning sums of squares to mean pairwaise distance (MPD) matrices as beta diversity measurement. Different MPD matrices were establishing for taxonomic distance (TD), phylogenetic distance (PhD) and functional distance (multi-trait approach (MT) and single-fuctional trait). Variance were studied defined each site at two scales, 64 cells data set (C) and 5 subplots data set (S) of 39 sites.

These results show a higher variance source at finer scales, suggesting a larger turnover effect at these scales than regional scales. One again the importance of heterogeneity environmental or the interactions determines the local species composition. Both processes promote that species appear in different niches within a relatively reduced surface area and therefore the beta diversity be more variable at these scales than broad scales.

Functional and phylogenetic divergence-convergence

Significantly convergence and divergence were found depending on work scales (i.e. cell, subplot or plot scale) and null model using to build the random communities (i.e. species consideration from plot pool – NM1 – or from regional pool – NM2 –). Significant trait convergence (SES < -1.96) was the dominant pattern at plot scale for all single-trait and multi-trait approach, while the divergence pattern was less patent at this scale ranged from 1 (LDMC trait) to 9 plots (SLA trait) with coexisting species more different than the random expected. Significant trait divergence (SES value > -1.96) had a considerable importance for most functional traits to subplot scale when the plot pool species were used to build the random communities. Maxima height traits showed a significantly convergence pattern for most plots at this scale. When we used the regional species pool at subplot scale divergence pattern was less evident, except in seed mass trait. No significantly convergence or divergence was detected for most traits at cell scale irrespective of whether is deemed the plot or the regional pool species. Nevertheless a considerable number of plots showed a divergence pattern for seed mass at cell scale.

Two findings can be released from these results. First the significance of functional traits in the species coexistence and second the scale-dependence of the processes involved in the assembly coexistence (Mason *et al.*, 2011; de Bello *et al.*, 2013). Our study showed as an increased in the work area (i.e. plot scale – 20 x 20 m) implies a higher convergence in most functional traits. This support the idea that effects of environmental filters are more patent at broad scales (Weiher & Keddy, 1995). When the work area is reduced at subplot scale (2.4 x 2.4 m) the traits divergence between species was the more frequent pattern. This is according to some works that have found divergence at small scales and under similar environmental conditions (Stubbs & Bastow Wilson, 2004; de Bello *et al.*, 2013) and supports the limiting similarity hypothesis for most functional traits. Nevertheless plant height trait was characterized by a convergence at this scale (Fig. 3), so the species at each subplot seem showed similar height. In high mountain the cold represents a strong abiotic filter and species height represents an handicap to survival (Körner, 2003). Thus, it is not unusual that a convergent strategy is the norm in our community respect to the species height. Finally the convergence – divergence pattern was less patent at finest scales (cells of 0.3 x 0.3 m). In this case convergence and divergence pattern may be offset in view of the high heterogeneity showed at this scale (see Fig. 1 and above discussion).

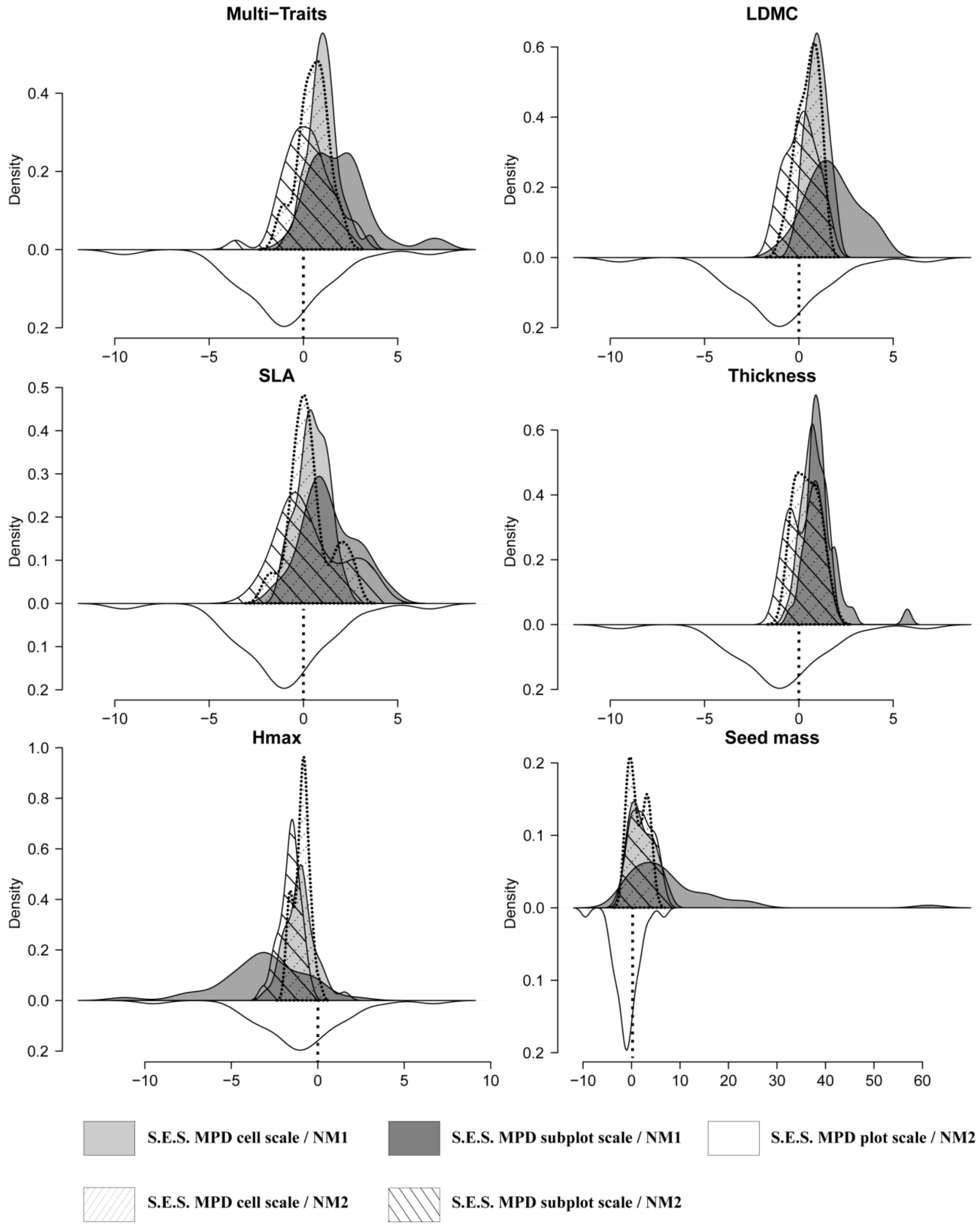

Fig. 3. Density plots of Standard Effect Size of MPD for each individual plant functional trait and multi-trait approach. Three different work scales (cell, subplot or plot scale) and two null models (simulated assemblages using plot pool –NM1– and regional pool –NM2–) are represented.

The responses of environmental predictors on S.E.S MPD were dependent on functional trait and null model considering (Table 1). Specifically, the altitude was more important at broad scales and when NM2 (species pool from regional pool) was used. This predictor was inversely correlated with SES MPD of multi-trait, SLA and Seed mass traits and thus the species convergence of these traits increased with the altitude.

Table 1. Correlation between Standard Effect Size (SES) of MPD and environmental predictors. SES MPD values are considered at three work scale (cell, subplot or plot scale) and under two null models (simulated assemblages using plot pool –NM1– and regional pool –NM2–). Only the significantly relationships are showed.

		Altitude		**Insolation**		**SOC**		**NT**		**pH**	
		MN1	MN2	MN1	MN2	MN1	MN2	MN1	MN2	MN1	MN2
Cell scale	MT	0,34									
	Hmax								-0,37		0,41
	Thickness						-0,42		-0,51		
	SLA		-0,46				0,43		0,61		-0,48
	LDMC										
	Seed mass									0,51	0,33

		Altitude		**Insolation**		**SOC**		**NT**		**pH**	
		MN1	MN2	MN1	MN2	MN1	MN2	MN1	MN2	MN1	MN2
Subplot scale	MT			-0,33							
	Hmax									0,32	0,51
	Thickness	-0,31					-0,53	0,36	-0,62	-0,53	
	SLA		-0,57				0,47		0,61	-0,44	-0,49
	LDMC									-0,38	
	Seed mass										

		Altitude	**Insolation**	**SOC**	**NT**	**pH**
		MN2	MN2	MN2	MN2	MN2
Plot scale	MT	-0,46				
	Hmax					
	Thickness			-0,54	-0,66	
	SLA	-0,6		0,5	0,62	-0,46
	LDMC					
	Seed mass	-0,38				

On the other hand edaphic predictors such as SOC, total N and pH gained importance at local scales. In general low pH levels implied a notorious divergence in the case of foliar thickness, SLA and LDMC. Besides low soil levels of total N and SOC led to divergence in foliar thickness but a convergence in SLA.

These findings show that the dominance of the assembly patterns is spatially dependent. Environmental filtering was expected at broader spatial scale where species coexist under particular conditions and share traits to adjust at these conditions. On the other hand the divergence was presented at finest scales suggested a niche differentiation. This pattern seems modulate the assembly community and the heterogeneity or interactions between species at finest scales are really important. This highlights the need for be careful when using predictive models of species distribution based on environmental conditions and species functional traits.

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SUPPLEMENTARY MATERIAL

Table S1. Functional traits for fifty six species studied: Hmax, plant length between the ground and the top of photosynthetic tissues; foliar thickness; SLA, specific leaf area; LDMC, leaf dry matter content; Seed mass. Values are shown as mean of individuals samples (Nº ind. samples) ± standard deviation.

Species	Nº ind. sampled	Hmax (mm)	Foliar thickness (µm)	SLA (mm^{2}·mg^{-1})	LDMC (mg·g^{-1})	Seed mass (µg)
Adenocarpus complicatus	10	789.4 ± 113.9	243.8 ± 54.3	10.2 ± 3.7	313.2 ± 40.3	44079.5 ± 4703.2
Agrostis rupestris	10	37.3 ± 9.4	142.4 ± 20.7	15.9 ± 1.1	376.9 ± 16.9	52.7 ± 18.3
Agrostis delicatula	70	20.5 ± 6.8	209.1 ± 42.9	11.4 ± 2.0	397.3 ± 44.5	63.7 ± 13.8
Arenaria queriodes	10	17.7 ± 5.1	174.5 ± 41.9	17.6 ± 5.6	327.2 ± 38.6	368.4 ± 51.2
Armeria caespitosa	90	16.2 ± 4.3	232.3 ± 37.8	15.1 ± 2.2	350.7 ± 34.9	641.7 ± 94.0
Arnoseris minima	10	11.1 ± 3.9	295.7 ± 35.5	28.2 ± 5.7	126.0 ± 14.3	123.1 ± 27.5
Biscutella valentina subsp. *pyrenaica*	20	33.4 ± 10.5	330.9 ± 68.4	11.8 ± 2.1	208.0 ± 20.3	1202.9 ± 231.7
Campanula herminii	10	19.9 ± 5.6	152.5 ± 21.3	24.2 ± 2.5	214.9 ± 17.6	60.4 ± 5.1
Cerastium ramosissimum	20	38.9 ± 7.2	258.7 ± 29.9	23.5 ± 4.2	162.9 ± 24.8	169.9 ± 17.3
Crocus carpetanus	10	123.8 ± 24.9	279.7 ± 22.7	7.7 ± 0.6	264.0 ± 20.1	5763.2 ± 786.3
Cytisus oromediterraneus	20	502.5 ± 120.8	224.5 ± 47.4	14.3 ± 2.2	258.2 ± 32.1	10761.8 ± 1387.1
Deschampsia flexuosa	80	27.6 ± 9.4	302.2 ± 57.2	7.7 ± 1.2	396.9 ± 30.7	617.1 ± 130.0
Dianthus sp.	10	15.8 ± 4.2	239.2 ± 42.5	13.0 ± 1.8	297.2 ± 17.1	877.6 ± 165.0

Table S1. Cont.

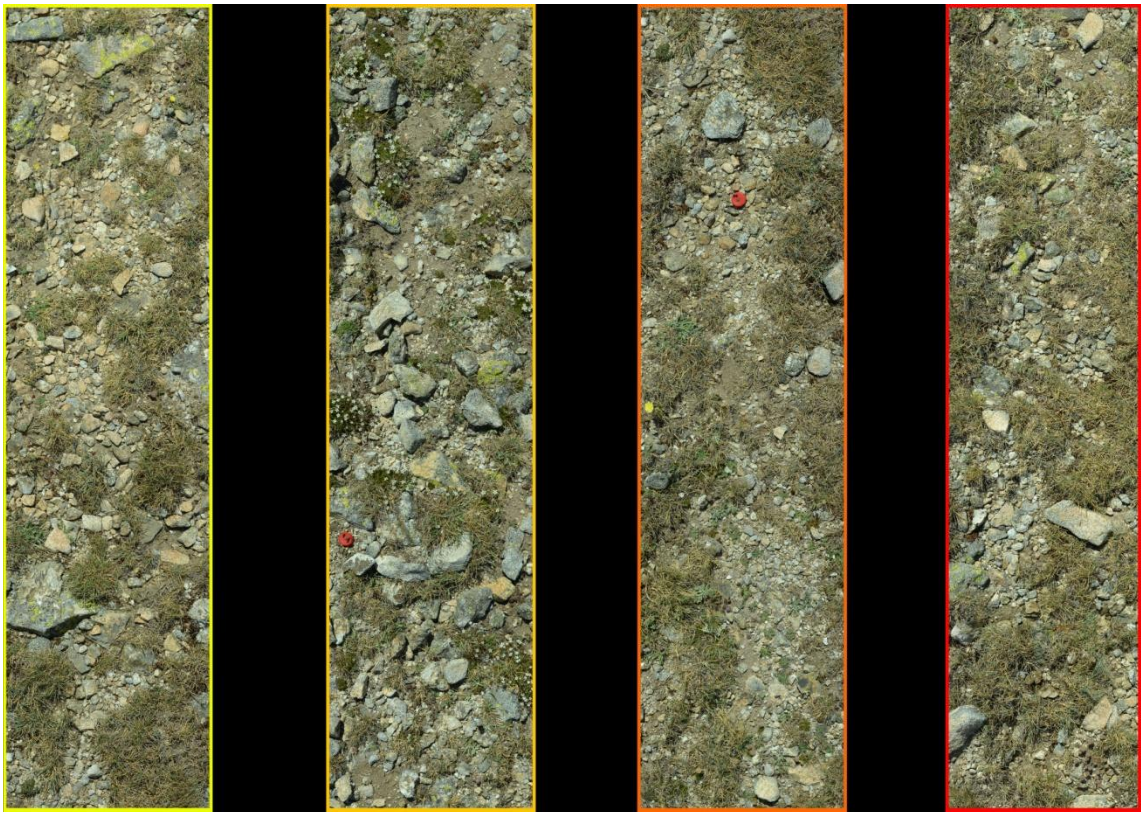
Dianthus lusitanicus	10	33.6 ± 10.9	409.5 ± 104.2	8.9 ± 1.3	273.6 ± 18.0	1038.2 ± 203.5
Erophila verna	10	1.9 ± 0.5	188.2 ± 33.6	25.2 ± 4.0	140.5 ± 24.3	38.7 ± 6.3
Erysimum humile subsp. *penyalarensis*	20	30.6 ± 11.4	209.0 ± 33.0	20.1 ± 4.3	201.4 ± 33.8	774.6 ± 128.1
Euphasia willkommii	10	29.2 ± 7.8	87.8 ± 19.7	22.9 ± 2.4	235.9 ± 31.5	115.1 ± 22.4
Festuca iberica	10	63.6 ± 17.3	146.8 ± 28.1	9.6 ± 1.1	383.1 ± 36.8	790.4 ± 107.3
Festuca curvifolia	90	32.4 ± 9.7	559.3 ± 101.0	4.3 ± 0.6	463.5 ± 40.4	710.6 ± 263.0
Gagea nevadensis	20	58.8 ± 14.4	227.6 ± 22.6	20.6 ± 2.2	170.4 ± 10.6	339.3 ± 61.4
Gentiana lutea	10	182.0 ± 60.5	268.2 ± 37.3	8.9 ± 1.0	267.3 ± 14.7	964.7 ± 243.6
Herniaria glabra	10	11.4 ± 3.4	236.0 ± 40.7	13.3 ± 1.8	212.1 ± 19.6	75.4 ± 7.2
Jasione crispa	80	15.0 ± 4.5	188.0 ± 36.9	18.4 ± 3.7	241.6 ± 27.6	49.6 ± 5.2
Juniperus communis subsp. *alpina*	80	233.1 ± 85.0	417.9 ± 68.7	6.5 ± 1.1	434.4 ± 46.6	10655.2 ± 2396.0
Jurinea humilis	90	7.0 ± 3.3	262.3 ± 38.0	10.1 ± 1.5	266.3 ± 34.9	7019.8 ± 1239.3
Koeleria crassipes	10	25.9 ± 3.8	170.0 ± 14.6	12.6 ± 0.6	390.7 ± 11.0	236.7 ± 37.1
Leontodon sp.	10	41.2 ± 10.7	234.8 ± 34.6	19.7 ± 3.0	158.7 ± 15.7	1054.0 ± 247.0
Leucanthemopsis alpina	20	18.0 ± 5.7	170.5 ± 43.2	17.3 ± 2.9	216.9 ± 25.2	150.7 ± 32.7
Linaria elegans	10	15.8 ± 6.6	314.4 ± 133.9	23.6 ± 3.2	118.3 ± 13.7	11.9 ± 1.1
Linaria saxatilis	10	17.7 ± 3.4	128.2 ± 30.5	21.5 ± 4.2	210.4 ± 22.4	88.9 ± 8.4
Luzula spicata	20	44.4 ± 9.7	255.4 ± 60.3	13.3 ± 2.6	305.8 ± 30.2	253.6 ± 34.1
Minuartia recurva	46	16.7 ± 7.0	111.2 ± 53.1	17.2 ± 2.8	340.2 ± 47.3	248.5 ± 37.9
Nardus stricta	20	85.3 ± 15.6	205.0 ± 31.3	6.8 ± 0.8	401.7 ± 17.2	239.0 ± 29.1
Paronichya polygonifolia	10	20.0 ± 7.9	129.0 ± 16.7	18.4 ± 3.2	315.1 ± 44.2	328.1 ± 39.8
Phyteuma hemisphaericum	10	22.2 ± 6.6	122.3 ± 6.9	18.1 ± 2.3	269.4 ± 17.4	73.0 ± 12.4
Pilosella argyrocoma	10	25.7 ± 4.9	288.6 ± 19.2	13.9 ± 1.7	254.3 ± 26.4	287.6 ± 49.9

Table S1. Cont.

Pilosella castellana	10	22.3 ± 13.4	199.9 ± 25.8	13.8 ± 1.3	261.9 ± 26.2	169.6 ± 36.7
Pilosella pseudopilosella subsp. *pseudopilosella*	10	12.7 ± 4.1	248.5 ± 24.1	13.5 ± 1.4	234.6 ± 18.5	182.0 ± 21.7
Pilosella vahlii	80	21.9 ± 8.3	287.9 ± 42.2	17.1 ± 2.8	201.0 ± 28.2	181.2 ± 18.4
Poa bulbosa	10	26.0 ± 6.1	64.8 ± 12.7	23.9 ± 7.6	285.6 ± 31.3	949.5 ± 232.7
Ranunculus bulbosus subsp. *aleae*	20	28.5 ± 8.4	227.0 ± 33.0	22.3 ± 3.4	173.8 ± 13.1	564.1 ± 175.5
Rumex angiocarpus	10	43.8 ± 19.6	400.6 ± 45.5	10.6 ± 1.5	189.3 ± 24.4	335.6 ± 65.1
Scleranthus perennis	10	15.0 ± 4.7	136.4 ± 36.9	25.7 ± 3.2	185.3 ± 9.3	628.7 ± 67.9
Sedum amplexicaule	10	21.7 ± 7.8	653.2 ± 239.6	10.5 ± 1.3	154.2 ± 10.4	9.7 ± 1.7
Sedum brevifolium	10	13.1 ± 5.4	1588.5 ± 213.6	10.3 ± 2.2	87.5 ± 20.0	68.4 ± 8.9
Sedum candollei	10	12.7 ± 3.0	842.9 ± 93.2	19.3 ± 2.7	87.8 ± 9.8	21.6 ± 2.4
Senecio carpetanus	60	313.8 ± 89.6	486.7 ± 79.9	10.3 ± 1.5	189.6 ± 29.3	2781.8 ± 313.2
Silene ciliata	80	19.6 ± 5.1	353.1 ± 70.8	14.1 ± 1.9	207.0 ± 24.2	351.5 ± 49.5
Solidago virgaurea subsp. *fallit-tirones*	10	23.5 ± 6.0	218.0 ± 17.7	12.3 ± 1.0	286.1 ± 16.5	862.0 ± 159.4
Spergula morisonii	10	7.3 ± 2.3	99.9 ± 34.1	30.5 ± 7.2	139.3 ± 29.1	240.7 ± 21.8
Taraxacum sp.	10	22.2 ± 6.3	181.4 ± 14.6	20.7 ± 2.3	193.8 ± 18.8	342.0 ± 102.5
Teesdalia nudicaulis	10	6.0 ± 3.7	131.3 ± 35.5	30.3 ± 8.1	142.3 ± 29.1	315.1 ± 46.1
Thymus praecox subsp. *penyalarensis*	10	21.1 ± 4.8	197.7 ± 16.0	14.9 ± 1.7	320.0 ± 27.4	197.5 ± 37.5
Veronica fruticans subsp. *cantabrica*	10	17.1 ± 5.2	234.7 ± 22.6	12.9 ± 1.8	308.7 ± 29.6	157.1 ± 21.7
Veronica verna	10	25.4 ± 12.7	199.6 ± 81.2	24.7 ± 3.4	201.1 ± 29.9	153.5 ± 15.3
Viola sp.	10	35.9 ± 8.2	279.1 ± 48.6	25.2 ± 2.8	169.2 ± 15.8	292.8 ± 33.9

IV

Plant to plant networks in a Mediterranean high mountain community: dominance of Nestedness over Modularity



Pescador, D., Iriondo, J.M. and Escudero, A.

Manuscrito inédito

ABSTRACT

Alpine communities, where harsh conditions lead to species co-occurrence within plant-patches structures, represent an adequate system to establish ecological networks which may shed light on the community assembly patterns. Previous efforts have been aimed at study of nestedness or modularity properties under a large-spatial-scale framework but not at smaller scale such as the patch scale where real interactions occur. In this study, we selected 37 grassland communities, distributed along a mountain range in Central Spain. In each location, we identified all plant species within vegetated patches along 25 m of transect lines and combined the data into a plant-patch bipartite network. Nestedness and modularity were estimated and related with different environmental and structural predictors. Our results showed a nested and non-modular pattern for most of our networks. Both indices were indirectly correlated and these in turn were depending on network connectance. Nestedness was positively related with mean patch area and negatively with the species per plot and total area pasture while modularity responded positively to species by plot and total pasture area. This study suggests that multiples processes operate simultaneously in the patch structure and dynamics of Mediterranean high mountain communities. The results support the idea that nestedness domain in early and intermediate stages where few species co-occur and positive interactions are the dominant process. As niches segregation by competitive exclusion limits the number of coexisting species in the same patch new modules are gradually consolidated increased the modularity. Modularity may be promoted in larger pasture where more niches and microhabitats allow the establishment of new species. In contrast, patch size heterogeneity represents a particular case of nestedness closely linked to species-area relation. Assessing the importance of the relationship between nestedness and modularity may provide a better understanding of assembly community.

INTRODUCTION

The search for patterns/structures in species assemblages and meta-communities represents a main and classical goal of community ecologists. In this sense, relationships between species in a community usually converge on network structures, which are dependent not only on the nature of the interaction between them but on ecological factors and evolutionary history (Bascompte & Jordano, 2007). Observation of not only species spatial patterns but main architectural properties of networks (i.e. connectance, nestedness and modularity) is basic to understand the ecological processes behind assembly and stability of communities (Mcintire & Fajardo, 2009; Thébault & Fontaine, 2010).

Nestedness was originally presented in the context of insular biogeography (Patterson & Atmar, 1986; Atmar & Patterson, 1993) by capturing information structures from binary matrices with rows as species and columns as islands. Lately nestedness analysis was extended to bipartite interaction networks, such as plant-animal (Bascompte *et al.*, 2003; Lewinsohn *et al.*, 2006), host-parasitism (Poulin, 1996; Graham *et al.*, 2009) or plant-plant (Burns, 2007; Verdú & Valiente-Banuet, 2008). Current knowledge on the topic highlights that nested patterns seem to be the norm in geographic and ecological networks (Bascompte *et al.*, 2003). This pattern occurs when species compositions of poor sites form a nonrandom subset of species-rich sites (Patterson and Atmar 1986). In this sense, in a perfectly nested community any species found in a particular site will also be found in all sites with equal or higher species richness while species absent in that site will never be found in sites with lower species richness rich (see Ulrich and Almeida-Neto 2012 for a detailed review of the meaning of nestedness in ecology). Complementarily modularity represents another common pattern that a network can exhibit, although it has been less frequently investigated probably because of its complexity and lack of computational tools (Montoya *et al.*, 2006). Nevertheless recent advances (Newman & Girvan, 2004; Guimera, Roger & Amaral, Luis A Nunes, 2005; Barber, 2007) have increased its evaluation in different types of ecological networks (Olesen *et al.*, 2007; Rezende *et al.*, 2009; Stouffer & Bascompte, 2011; Carstensen *et al.*, 2012; Montesinos-Navarro *et al.*, 2012). Modularity is characterized by the existence of non-overlapping subsets of nodes, known as modules, more connected among themselves than with the nodes of other subsets. Despite the relative importance of nestedness and modularity as basic underlying architectures in community assembly the relationship between them has been poorly studied (but see Lewinsohn, 2006; Carstensen & Olesen 2009; Presley *et al.* 2010; Olesen *et al.* 2011; Montesinos-Navarro *et al.* 2012; Dalsgaard *et al.* 2013). Recently Fortuna *et al.* (2010) analyzed the relationship between nestedness and modularity for random matrices generated from real networks and found an increasing correlation between both patterns with a decreasing connectance. Consequently, only networks with low connectance may show simultaneously nested and modular patterns, reaffirming but limiting the findings of Olesen *et al.* (2007). Unfortunately the balance between both patterns in real assemblage has to our knowledge rarely tackled.

In harsh habitats, such as desert and alpine environments, plants may occur in biphasic spatial structures, with plants confined into patches surrounded by a bare ground matrix (Kikvidze, 1993; Nuñez *et al.*, 1999). Such a pattern can be described by bipartite networks considering a set of patches as a meta-community where species occur and interact in each patch (Callaway *et al.*, 2002; Callaway, 2007; Brooker *et al.*, 2008; Kikvidze *et al.*, 2011). This approach leads to the construction of real plant to plant networks because we can be sure that plants living in a patch are really interacting.

This is completely different from networks arisen from the detection of co-occurring patterns in sample cells of different sizes. The packing process and distribution of species within patches may not be random and can exhibit different patterns and dynamics (Escudero *et al.*, 2004). Similarly to island biogeography (Wallace, 1881; MacArthur & Wilson, 1967; Diamond, 1975) patch characteristics, such as size, isolation and stage in the history of a patch are linked to the assembly process (Triantis *et al.*, 2008), although biotic interactions also play a role in defining species coexistence in this systems (Callaway *et al.*, 2002; Kikvidze *et al.*, 2005; Butterfield *et al.*, 2013). Thus, in this biphasic system, with species organized in patches of vegetation, we hypothesized that four basic sets of patterns may be feasible from the plant to plant interactions that species conform in harshful environments. This results from the factorial combination of nestedness and modularity patterns (Fig. 1). Under a situation with nestedness as dominant pattern, the meta-community (i.e. patch to patch structure) follows a dynamic and gradual process where the species that shape the patches are always the same and the rest of species arrange in a sequential order according to ecological or stochastic drivers (Ulrich *et al.*, 2009). Ecological drivers include: different colonization (Patterson, 1990) or extinction (Patterson & Atmar, 1986; Lomolino, 1996) rates along gradients, nested habitats (Wright & Reeves, 1992) or selective environmental tolerances linked to environmental harshness (Blake, 1991). Besides, neutrality process (Ulrich & Zalewski, 2007) and passive sampling (Cutler, 1994) may also cause nested subset patterns. In a modular pattern, environmental heterogeneity, functional or phylogenetic clustering and divergent selection favor interaction among distinguishable modules of species (Lewinsohn *et al.*, 2006; Olesen *et al.*, 2007; Parter *et al.*, 2007). A scenario where both patterns are present is more complex and may be found in a meta-community responding to small scale heterogeneity or consisting of patches in different successional states, i.e. there are a few species shaping the patches with several pioneers establishing the patch and other species replacing them along time. This process may result in several differentiated modules with a nested structure each. Finally a random organization of species is possible if there are not any assembly rules and structures are neutral and depend exclusively on dispersal and not plant to plant interactions (Hubbell, 2001). These scenarios are neither exclusive or independent on a site and although global parameters and structure of networks remain stable over time (Alarcón *et al.*, 2008; Petanidou *et al.*, 2008; Dupont *et al.*, 2009; Díaz-Castelazo *et al.*, 2010), biotic and abiotic factors can influence nestedness and modularity patterns (Rezende *et al.*, 2009; Rico-Gray *et al.*, 2012). As recently highlighted by Ulrich and Almeida-Neto (2012) nestedness variation between sites involve differences in species richness and ecological gradients (i.e. species and sites characteristics). Therefore correlations between site and/or species nested ranks and independent predictors has been proposed to identify the underlying mechanisms of nestedness (Azeria & Kolasa, 2008; Sasaki *et al.*, 2012). Overall, nestedness and modularity patterns are directly driven by local conditions and respective responses along structural and environmental gradients may be recorded.

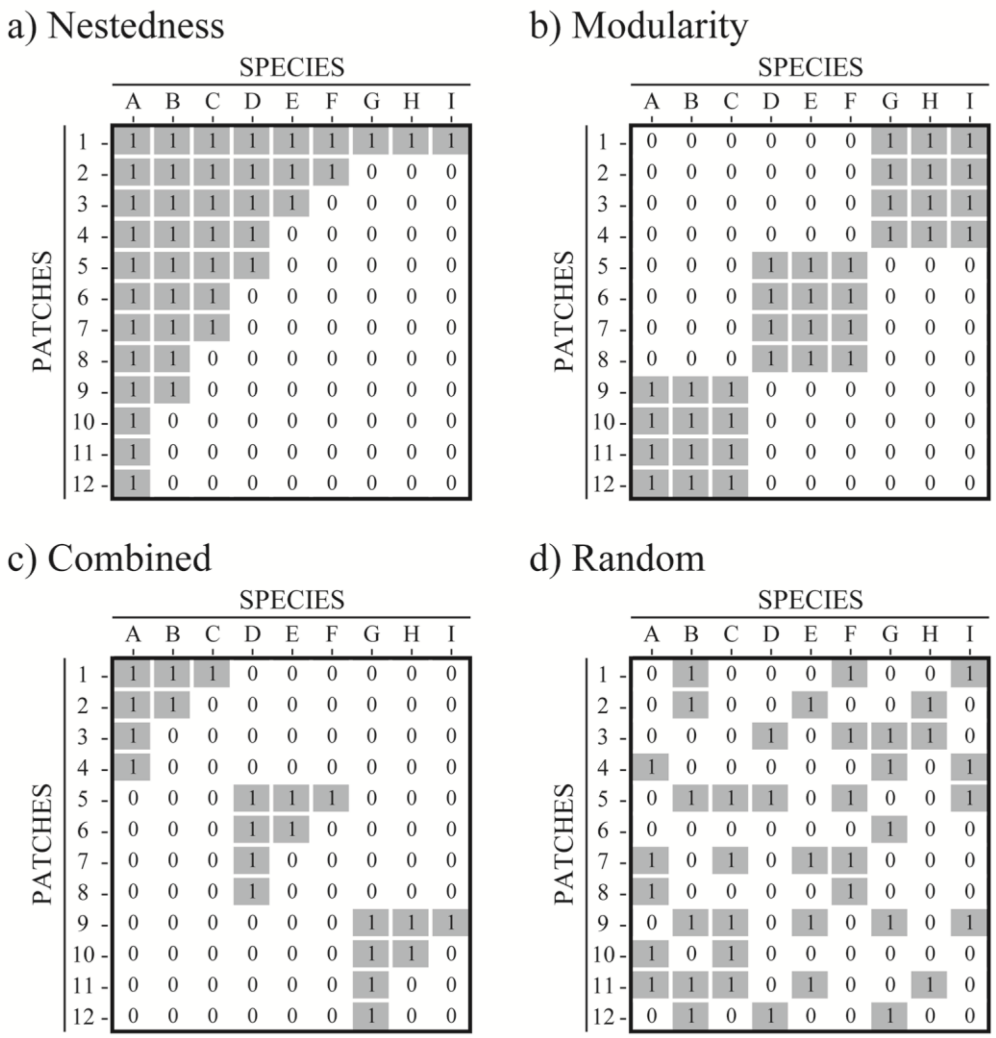


Fig. 1. Theoretical presence-absence matrices showing the four basic patterns hypothesized for plant to plant ecological networks in a biphasic realized community. a) Only Nestedness. b) Only Modularity. c) Combination of nestedness and modularity. d) Random pattern. Each matrix represent the species (in columns) co-occurring in different vegetated patches (rows).

Here we evaluated nestedness and modularity patterns, as well as their relationship with connectance, in 37 plant-patch interactive networks located in a Mediterranean high-mountain grassland subject to environmental and structural gradients but without patent species turnover. We hypothesized that plant to plant interactions and the subsequent nestedness pattern would be dominant and that with increasing environmental severity, species of each meta-community would tend to aggregate in vegetation patches following a hierarchical order. We also hypothesized that only in the gradient edges, where species of other neighboring habitats may co-occur, modular patterns would be likely to be found. Thus, using specific metrics to estimate both network properties and appropriate null models, we aimed to (1) identify and compare the dominant patterns of the four theoretically feasible (i.e. nestedness and modularity) in each plant-patch network, (2) assess the response of each pattern along environmental and structural gradients.

MATERIALS AND METHODS

Study site and data set

This study was carried out in the National Park of Sierra Guadarrama, a mountain range located in the center of the Iberian Peninsula about 70 km north of Madrid, Spain. The climate in this region is Mediterranean with an annual mean temperature of 6.4ºC and an average annual precipitation of 1350 mm concentrated from early October to late May (Navacerrada pass weather station, 40° 46' N, 4° 19' W, 1860 m a.s.l.). A pronounced dry season (< 10% of total annual rainfall) occurs from June to September, which is more extreme at lower altitudes (Giménez-Benavides *et al.*, 2007).

The timberline goes up to 1900 – 2000 m a.s.l. and is dominated by pines (*Pinus sylvestris* L.) interspersed in a shrub matrix formed by *Cytisus oromediterraneus* Rivas Mart. & al. and *Juniperus communis* subsp. *alpina* (Suter) Čelak. Above this limit a psychroxerophilous grassland community is found, which has phytosociologically ascribed to *Hieracio myriadeni-Festucetum curvifoliae* (Rivas-Martínez, 1963). This community forms the top alpine vegetation stage in Guadarrama, and it is topographically isolated and characterized by short pasture with several creeping, chamaephytes, caespitosous grasses, lichens and mosses. Plants are structured in small ellipsoidal-shaped patches that usually do not exceed 30 cm in diameter, mainly composed of *Festuca curvifolia* Lag. *ex* Lange, where different plant endemics can be found, such as *Pilosella vahlii*, *Minuartia recurva* or *Armeria caespitosa*, as well as arctic and alpine relicts, such as *Agrostis rupestris* and *Phyteuma hemisphaericum*.

To perform the study we selected 37 localities dominated by pastures of *Festuca curvifolia* and as far as possible poorly grazed. Localities were distributed along an altitudinal gradient (between 1940 – 2428 m a.s.l.), with different orientation, slope, pasture and shrub cover. In each locality we established a square plot of 20x20 m where plant species cover and percentage of bare soil was estimated visually. Altitude and orientation were determined using a GPS receiver (Garmin Colorado-300) and a clinometer was used to quantify the slope (Silva Clinomaster CM-360%, LA). Orientation and slope values allowed us to estimate the insolation coefficient by the Gandullo´s method (Gandullo, 1974; Pérez, 1997). We measured the total area of pasture where each plot was localized using ortho-photos taken in 2009 from Aerial Orthophotography Plan (PNOA; http://www.ign.es/PNOA/) resource and ArcGis 10.2 software (ESRI, 2011).

Within each site we established four transect lines of 6.25 m (148 transect lines in total) parallel to the maximum slope and distributed throughout the plot. Every vegetation patch in contact with the transect line was measured in their two maximum dimensions and all species locate inside it were identified. We combined the data of the four transects into one single qualitative 0-1 matrix for each site, with species represented by columns and patches in rows, to establish 37 plant-patch bipartite networks. Each matrix cell is 1 when the species that denotes this column is present in the patch that denotes the row, so cells with 1´s in a row represent species co-occurring in the same vegetation patch.

Network architecture parameters

For each of 37 qualitative matrices we estimated three network parameters to describe connectance, nestedness and modularity. Connectance represents the proportion of the links observed in our network in relation to the total number of possible links (i.e. cells in the matrix; Dunne *et al.* 2002).

The search of nested patterns was done in accordance to the packaging of species, i.e. by rows, and thus considering whether assemblages in species-poor patches constitute subsets of richer ones. A recent and consistent nestedness metric based on overlap and decreasing fill (NODF; Almeida-Neto *et al.* 2008) was used to measure the nestedness value of each network. This metric allows to discriminate nestedness for rows and columns independently and overcomes some problems set out by previous metrics (see Ulrich *et al.* 2009 for more details). Overall, NODF metric is not affected by matrix size or shape nor is correlated to matrix fill and limits the chance of overestimating nestedness. This index ranges from 0 for a non-nested network to 100 for a perfectly nested network and is calculated as the ratio of presence overlap for each pair of columns and for each pair of rows in an ordered matrix.

The degree of modularity of each interaction network was assessed through the index M_b proposed by Barber (2007) for bipartite networks, chosen as the most precise modularity metric (Thébault, 2013). M_b is formulated as follows:

$$M_b = \sum_{i=1}^{N} \left[\frac{L_i}{L} - \left(\frac{K_i^C \cdot K_i^R}{L^2} \right) \right] ,$$

where N is the number of modules in the network, L is the total number of links in the network, L_i is the number of links between all nodes within module i, K_i^C and K_i^R are the sum of the degrees of all the C-nodes and all the R-nodes respectively within module i. A simulated annealing algorithm was used to identify and count number of modules that maximized the modularity after randomly changes (i.e. moving nodes, merging and splitting modules; Guimera & Amaral 2005a; Guimera & Amaral 2005b). Therefore M_b defines how good is the partition in modules of our network, with values between 0 (when the distinction between modules is diffuse) and 1 (when the modules are perfectly defined).

The statistical significance of nestedness and modularity was estimated by comparing the observed $\mathrm{NODF_r}$ or M_b values for each network against random expectations. Several null models have been proposed and the use of the one or another represents a challenge with the trade-off between type I and type II statistical errors (Gotelli, 2001; Ulrich & Gotelli, 2007; Gotelli & Ulrich, 2012). We used two relatively-constrained null models to attempt to minimize type I and type II statistical errors. The first null model was the Fixed row – Equiprobable column null model (FE) that preserves total number of occurrences and row totals (i.e., species by patch) but allows columns total (i.e., species occurrence frequencies) vary freely (Patterson & Atmar, 1986; Gotelli, 2000). The second was the Fixed row – Proportional columns null model (FP), which is more restrictive. It maintains total number of occurrences and row totals, and uses column marginal frequencies as probabilities to simulate original species frequencies. These null models limit the number of species by patch controlling the relationship between patch size and species richness within each patch but relaxes species frequency to allow the probability of each species occurrence to be the same (i.e FE null model) or to simulate original species occurrence (i.e. FP null model). We did not use the most constrained Fixed row – Fixed column null model (FF), that maintains both row and column totals, because large type II statistical errors have been described using NODF metric with this null model (Ulrich & Gotelli, 2013). Significance was estimated with a Monte-Carlo procedure for each network, metric (i.e. $\mathrm{NODF_r}$ or M_b) and null model (i.e., FE and FP), that is 37 networks x 2 metrics x 2 null models. First 1000 null matrices were obtained from the original matrix using the corresponding null models algorithms. Second, the metric value was estimated for each random matrix.

Finally, the P-value was defined by counting the number of random matrices that showed a metric value equal or higher than the value obtained with the original matrix and dividing it by the number of random matrices (n=1000). When no random matrices showed a higher metric value than that of the original matrix, we established $P<0.001$

Responses of network parameters across gradients

To determine what are the ecological drivers underlying nestedness and modularity patterns in this sort of plant to plant networks we assessed their response to environmental (altitude and insolation) and structural (species by site, shrub cover by site, mean patch area and total pasture area) non-correlated predictors through generalized lineal models (GLMs). We considered $NODF_r$ and M values as dependent variables and Gaussian family as error distribution. Note that we considered the NODF or M values regardless of whether they were significantly different from the null models since these values are intrinsic to each network. A full set of GLM models was carried out through combinations of all predictors. These models were ranked by rescaling of Akaike Information Criterion (AICc) value with respect to the model with the minimum AICc ($\Delta AICc = AIC_i - AIC_{min}$). Models with $\Delta AICc < 2$ were considered to be indistinguishable (Burnham & Anderson, 2002). The best explicatory model was defined through Akaike weights (w_i), estimated through ΔAICc according to Burnham and Anderson (2002). Finally the relative importance of each predictor (w+) was evaluated by summing of w_i values of models where the predictor of interest appeared.

Structural Equation Modeling (SEM) were also conducted to respectively test not only the direct causal relationships but also indirect that best explained the nestedness and modularity patterns (Shipley, 2002; Iriondo *et al.*, 2003; Schumacker & Lomax, 2004). We considered the results of GLMs as previous causal hypotheses to build the path diagram with the following relationships among variables: 1) nestedness is directly affected by mean patch area, species by plot and total area pasture, 2) modularity is directly determined by mean patch area, species by plot and total area pasture, 3) mean patch area and total area pasture indirectly effects nestedness and modularity through effects on species by plot. We propose a correlation between nestedness and modularity based on Fortuna *et al.* (2010) findings. The relative effect of one variable upon other, represented by an arrow in the path diagram, was evaluated through standardized path coefficients. These coefficients were calculated by the maximum likelihood method, as recommended for small sample size (see Shipley 2002 for details) . The significance of each individual coefficient was tested using multivariate Wald test. The initial hypothesis of the model (i.e., whether the model fits the data) was tested by a χ^2 goodness-of-fit test that compares the observed and predicted covariances. Non-significant χ^2 corresponds to a model that fits the data and where the pattern of covariance observed and predicted by the hypothesis are equal. Additionally the goodness-of-fit index (GFI) and the Bentler and Bonett´s normed-fit index (NFI) were also considered as goodness fit indices of the model. Both indices range from 0 to 1 and values above 0.95 indicate a good fit (Tanaka, 1987).

Connectance was calculated directly with R environment software (R Development Core Team, 2010) through *networklevel* function in bipartite package (Dormann *et al.*, 2008). Functions *oecosimu* and *commsimulator* in vegan R package (Oksanen *et al.*, 2013) were used to calculate the observed NODF_r values and simulate the random communities to estimate the significance. Modularity index was calculated in the software MODULAR (Marquitti *et al.*, 2013). All GLM models were built with MuMIn R package (Kamil, 2013). SEM analysis was carried out in AMOS v22 (SPSS, Chicago). Prior to theses analyses total area pasture was log-transformed to approximate the normal distribution.

RESULTS

A total of 5629 patches and 48 species were recorded in 925 m of transects distributed in 37 plots along an altitudinal gradient in Sierra de Guadarrama. The mean number of species by patch was 3 and ranged from 1 to 9 species. *Festuca curvifolia* was the dominant species, being present in all plots and in a total of 4491 patches (79,7 %), followed by *Sedum brevifolium* (1311 patches in 31 plots) and *Jurinea humilis* (1240 patches in 35 plots).

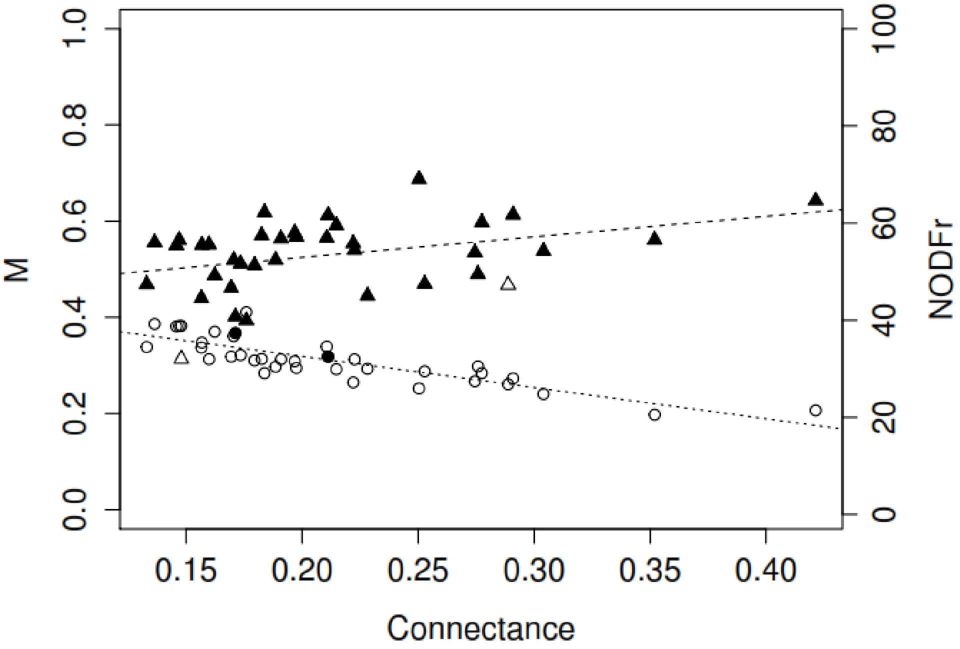


Fig. 2. Variation of modularity (M; circles) and nestedness (NODF; triangles) with the connectance for each plant-patch network studied. Significant pattern values according to fixed row-proportional columns (F-P) null model are represented by solid symbols. Dashed lines summarize the regression lineal model slopes between each pattern and connectance.

Most of our networks showed a nested pattern and lacked a modular pattern (Table 1). Modularity and nestedness values were negatively but significantly correlated (R = -0.5, p-value = 0.002), while connectance was positively correlated with $NODF_r$ values (R = 0.37, p-value = 0.02) and negatively with modularity (R = -0.85, p-value < 0.001;

Fig. 2). NODF_r values ranged from 32 (*Peña Cabra* plot) to 69 (*Hermana Menor*) with a mean value of 53.4. All plots were significantly nested by rows using the *F-E* null model, and most of them (35) were nested when the *F-P* null model was used. Modularity pattern was less evident with a mean modularity value of 0.31 ranging from 0.2 (*Los Horcajos*) to 0.41 (*Flecha*). No plots showed significantly modular patterns under the *F-E* null model while only two plots (*Bola del Mundo* and *Citores*) were significantly modular when *F-P* random expectations were considered.

Table 1. Descriptive statistics of each plant-patch network located across Sierra de Guadarrama range. Number of species (Sp.) and patches (P) per plot, connectance (C), nested overlap and decreasing fill for the overall matrix by rows ($NODF_r$), number de modules (N) and modularity value proposed by Barber (2007; *Mb*) are provide. Asterisks denote the significance of each $NODF_r$ and *Mb* value based on two null models: fixed rows-equiprovable columns (F-E) and fixed row-proportional columns (F-P). Significance levels are: *** p < 0.001 and ** p < 0.01.

Id.	Site	Sp	P	C	NODFr	P - Value		N	*Mb*	P - Value	
						F-E	F-P			F-E	F-P
1	Asomate-Hoyos	15	87	0.14	56.0	***	***	7	0.39		
2	Bola del Mundo	15	188	0.17	40.8	***	***	5.1	0.37		***
3	Bola del Mundo Oeste	13	180	0.15	56.5	***	***	7.8	0.38		
4	C. Bailanderos	11	119	0.29	47.3	***		6.8	0.26		
5	C. Guarramillas	11	221	0.22	54.4	***	***	5.8	0.31		
6	Cabeza de Hierro Mayor	9	152	0.29	61.7	***	***	5.8	0.27		
7	Cabeza de Hierro Menor	11	125	0.18	57.4	***	***	7.2	0.31		
8	C. de la Najarra	13	186	0.19	52.4	***	***	6.2	0.30		
9	C. de Peña Vaqueros	9	183	0.28	60.1	***	***	6.4	0.28		
10	C. Tirobarra	20	191	0.13	47.4	***	***	7.3	0.34		
11	C. de Valdemartín	11	177	0.30	54.2	***	***	6.7	0.24		
12	C. de las Zorras	11	114	0.25	47.5	***	**	7.1	0.29		
13	Cerro Minguete	14	175	0.17	51.6	***	***	7.8	0.32		
14	Citores	14	116	0.21	61.6	***	***	5.6	0.32		**
15	Flecha	11	155	0.18	39.9	***	***	5.8	0.41		
16	Guarramillas	10	132	0.28	49.5	***	***	5.2	0.30		
17	Hermana Mayor	7	119	0.42	64.6	***	***	4.3	0.21		
18	Hermana Menor	10	127	0.25	69.0	***	***	5.1	0.25		
19	La Maliciosa	14	126	0.16	55.5	***	***	7.5	0.31		
20	La Najarra	13	133	0.19	56.8	***	***	7.3	0.31		
21	Las Cerradillas	12	216	0.20	57.2	***	***	7.5	0.29		
22	Loma Noruego	13	167	0.22	55.8	***	***	5.2	0.26		
23	Los Claveles	9	134	0.27	53.9	***	**	5.5	0.27		
24	Los Horcajos	11	194	0.35	56.5	***	***	4.9	0.20		
25	Navacerrada	12	130	0.21	59.5	***	***	6.7	0.29		
26	Nevero	10	141	0.21	56.9	***	***	6.6	0.34		
27	Nevero Este	13	172	0.16	49.2	***	***	7.8	0.37		
28	Nevero Oeste	12	150	0.17	52.4	***	***	7.6	0.36		
29	Peña del Aguila	15	242	0.17	46.6	***	***	7.3	0.32		
30	Peña Bercial	12	195	0.23	45.0	***	**	5.4	0.29		
31	Peña Cabra	17	101	0.15	32.0	***		6.7	0.38		
32	Peñalara Norte	17	136	0.16	44.5	***	***	6.8	0.34		
33	Pto. de las Canderuelas	15	130	0.18	51.3	***	***	6.8	0.31		
34	Pto de Malagosto	16	118	0.16	55.5	***	***	6	0.35		
35	Pto. del Pasapan	10	125	0.20	58.0	***	***	6	0.31		
36	Reajo Alto	12	149	0.18	62.2	***	***	5.8	0.28		
37	Valdemartin	14	123	0.15	55.4	***	***	7.1	0.38		

The best and more parsimonious model (smallest AICc) selected for nestedness values explained 35% of the variance found and included three variables: species per plot, mean patch area and total pasture area. Specifically, species per plot, negatively related to nestedness, and mean patch area, positively related, were the most important predictors based in the relative importance (W_r = 1), while total pasture area (W_r = 0.43) was less important. The best modularity model explained more than 24 % of the variance and contained species per plot and total pasture area as predictors, which were positively related to modularity and had a relative importance of 1 (table 2).

Table 2. Model selection for A) nestedness and B) modularity patterns ranged according to Akaike Information Criterion (AICc). Predictor variables describing each model are marked with a cross. Ins: insolation, R^2: coefficient of determination, ΔAICc: delta AICc (ΔAICc = $AIC_i - AIC_{min}$), w_i: Akaike weights, w+: relative importance of each predictor. For each predictor a average-estimate value is showed. Total area pasture has been log-transformed.

A) Nestedness

Models	Ins.	Total pasture area	Mean patch area	pH	Sp per plot	R^2	AICc	ΔAICc	W_i
1	-	X	X	-	X	0.35	247.3	0	0.426
2	-	-	X	-	X	0.3	247.4	0.09	0.407
3	-	-	X	X	X	0.32	249.2	1.87	0.167
Averaged-estimate	-	-1.07	**0.04**	-3.07	**-0. 8**				
Wr	-	0.43	1	0.17	1				

B) Modularity

Models	Ins.	Total area pasture	Mean patch area	pH	Sp per plot	R^2	AICc	ΔAICc	W_i
1	-	X	-	-	X	0.24	-119.8	0	0.504
2	X	X	-	-	X	0.27	-118.6	1.15	0.283
3	-	X	-	X	X	0.26	-118.1	1.72	0.213
Averaged-estimate	-0.15	**0.013**	-	0.023	**$4.5 \cdot 10^{-3}$**				
Wr	0.28	1	-	0.21	1				

These results were also supported by the SEM analysis (Fig. 3). Our final model showed good fit, with non-significant χ^2 (0.278, DF= 1, P = 0.6) and high values of GFI and NFI (0.99 in both cases). No significant indirect effects were found.

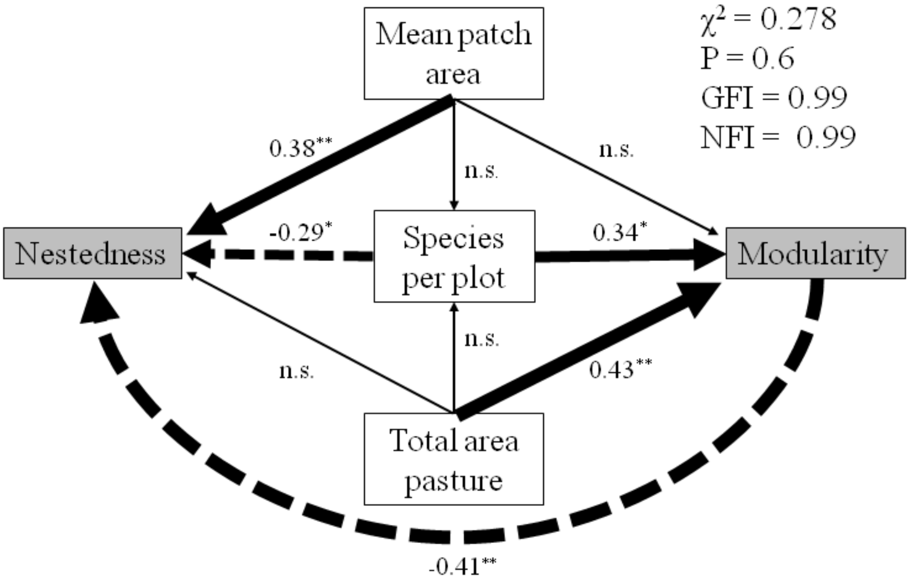


Fig. 3. Path model for nestedness and modularity determinants in plant-patch networks studied. Total area pasture has been log-transformed. Positive effects are indicated by solid lines and negative effects by dashed lines. Width of each arrow is proportional to the standardized path coefficient. Asterisks denote path coefficients that are significantly different from 0 according to multivariate Wald test ($p < 0.05$). Only significant standardized path coefficients are displayed. Goodness of fit statistics index (GFI), normed fit index (NFI), χ^2 and P for the model are showed.

DISCUSSION

We document the patterns underlying in 37 plant to plant networks mediated by patchy structures and their responses along environmental and vegetation structural gradients. Our results suggest that realized pattern at fine spatial scales of this harshful community is mainly driven by plant to plant interactions at the patch level following a nestedness process. Traditionally in biogeography, nestedness or modularity concepts have been applied to relatively large spatial scales (e.g. islands, ponds, forest or resources patches). At this large-spatial scales, we are dealing with co-occurring patterns but real biotic interactions are not specifically considered. In consequence the observed species patterns may reflect the effect of other factors, such as historical factors, environmental heterogeneity or dispersal ability and not necessarily the plant to plant interactions. In contrast, our approach explicitly considering vegetated patches as unit of measurement led to build networks where real biotic interactions occur and are directly linked to underlying patterns.

Our results showed a predominance of nested and non-modular pattern in most of our networks. That is, in the stressful environment of the highest areas of the Sierra de Guadarrama, species co-occur in patches with a nested pattern and hardly grouped in clearly defined modules. Since both patterns provide complementary information on assembly process (Thébault, 2013), these should be addressed jointly (Lewinsohn *et al.*, 2006). In our case both patterns were negatively correlated and both depended on network connectance. Significantly modular and nested patterns were only recorded simultaneously in two networks, which had relatively low connectance (maximum 0.21). This supports the findings of Fortuna *et al.* (2010), although connectance in most of our cases was not a determinant.

Null models are important in non-randomness pattern detection (Gotelli, 2001) and can assess the statistical significance of nestedness and modularity patterns, after discounting the effects of other factors such as differential species abundance and species richness - patch area relationships. In any case, regardless of the significance of these patterns against particular null models, the values of nestedness and modularity taken at each plot represent an intrinsic community property at such localities and obviously they can be characterized and studied. Our findings showed that both nestedness and modularity values in each plot are related with some ecological drivers and such relationships are direct without significant indirect relationships (see Fig. 3).

Plots with lower species richness had higher NODF and lower modularity values. This suggests that the arrival of new species in the realized assemblages leads to new independent modules and/or to further consolidation of the existing modules, reducing nestedness. In our particular system with a biphasic structure where plants are clumped in patches or stripes interspersed in a bare ground matrix, the restricted number of niches and the intense competitive exclusion (see the facilitation in the halo structure Pescador *et al.* 2014) probably are limiting the number of coexisting species in the same patch. Patterns of species coexistence in harsh habitats may comprise several dynamics steps based on positive and negative species interactions (Kikvidze 1993). In our system, Escudero et al. 2004 detected two contrasting stages, one conformed with pioneer plants together with most abundant species and the other dominated by creeping chamaephytes. In early and intermediate stages there are few species and positive interactions, such as facilitation, are the dominant processes in community assembly (Connell & Slatyer, 1977; Verdu *et al.*, 2009). Pioneer species modify microhabitat conditions and relax the environmental filter effects, shaping the assembly (Ellison *et al.*, 2005). Under this situation the species are hierarchically packaged and nestedness is the dominant pattern, reducing effective competition and increasing inter-specific coexistence (Bastolla *et al.*, 2009). An alternative explanation for the nested pattern may lay in passive sampling process, which entails that abundant species are more likely to colonize many patches than rare species and similarly larger patches have more species carrying capacity (Ulrich & Gotelli, 2007). Although meta-communities are typically characterized by this species/patches organization, the use of null models that constrain patch frequencies (i.e. null models FE and FP) control for this effect (Fischer & Lindenmayer, 2002; Ulrich & Gotelli, 2007). Therefore, the nested pattern observed in most of our plots must account for factors other than those associated to passive sampling process.

As new species establish in the plant community as a result of the microhabitat modifications performed by the pioneer species, above a certain threshold, competitive processes may arise as result of limiting resources. Based on the Lotka-Volterra competition model, classical ecological theory predicts that competitive species coexistence must occur in different niches (Silvertown, 2004; Kraft *et al.*, 2008). In our study, establishment of new species did not necessarily imply more modules, but it was found to be correlated with greater modularity. Consequently the establishment of new species may open new niches for other species to arrive, increasing connectance within modules rather than between modules.

The positive effect of the total pasture area could be related to the fact that establishment of new species in the community is dependent on the regional species pool. In our fragmented system, with “islands” of pasture at the highest elevations of Sierra de Guadarrama, the area of pasture defines, to some extent, plant species composition (Grashof-Bokdam, 1997). In our study, the plots found in larger pasture areas had higher modularity, although they did not have more species per plot directly (Fig. 3). When a new niche arises, the opportunity for new species reach it, coexist with present species and, consequently, increase modularity, depends largely, though not exclusively, on the species pool and its dispersal capacity, which are conditioned by the total area of pasture.

On the other hand the nestedness pattern was more evident when the mean patch area was higher. This predictor was correlated with the standard deviation of patch area (R = 0.71, P < 0.001) and thus plots with larger mean patch area have also patches of very different range sizes. Adapting the ideas of biogeography theory, patch size heterogeneity represents a particular case of nestedness (Lomolino, 1996; Wright *et al.*, 1997), which here may be subjected to larger patches contain more species co-occurring while smaller ones less. The reason could be a limitation of resources more evident in smaller patches or differential species area requirements, that is, species that co-occur in smaller patches are likely to be found everywhere while species with large area requirements only establish in larger patches. It is worth noting that nestedness pattern was not positively correlated with standard deviation of patch area because the plot with the highest patch area standard deviation (*Peña Cabra*) had the smallest nestedness value. This plot may be considered as an outlier with a singular species composition where the dominance of *F. curvifolia* is balanced by other species (e.g. *Festuca iberica*, *Silene ciliata* or *Deschampsia flexuosa, or Sedum brevifolium*) which occupy other niches and present individuals sizes very diverse.

The findings of the present study provide insights on processes that are determining the patch dynamics in communities structured in a biphasic mosaic of vegetated patches embedded in a bare ground matrix. Our approach, with species co-occurred in a same patch and configured real interaction networks, represents a conceptual advance in relation to biogeography approaches. The results are consistent with the main expectations about coexistence and species-area theories and represent, to the best of our knowledge, the first study where the evaluation of nestedness and modularity patterns on such a fine scale shed light on underlying mechanisms of community assembly.

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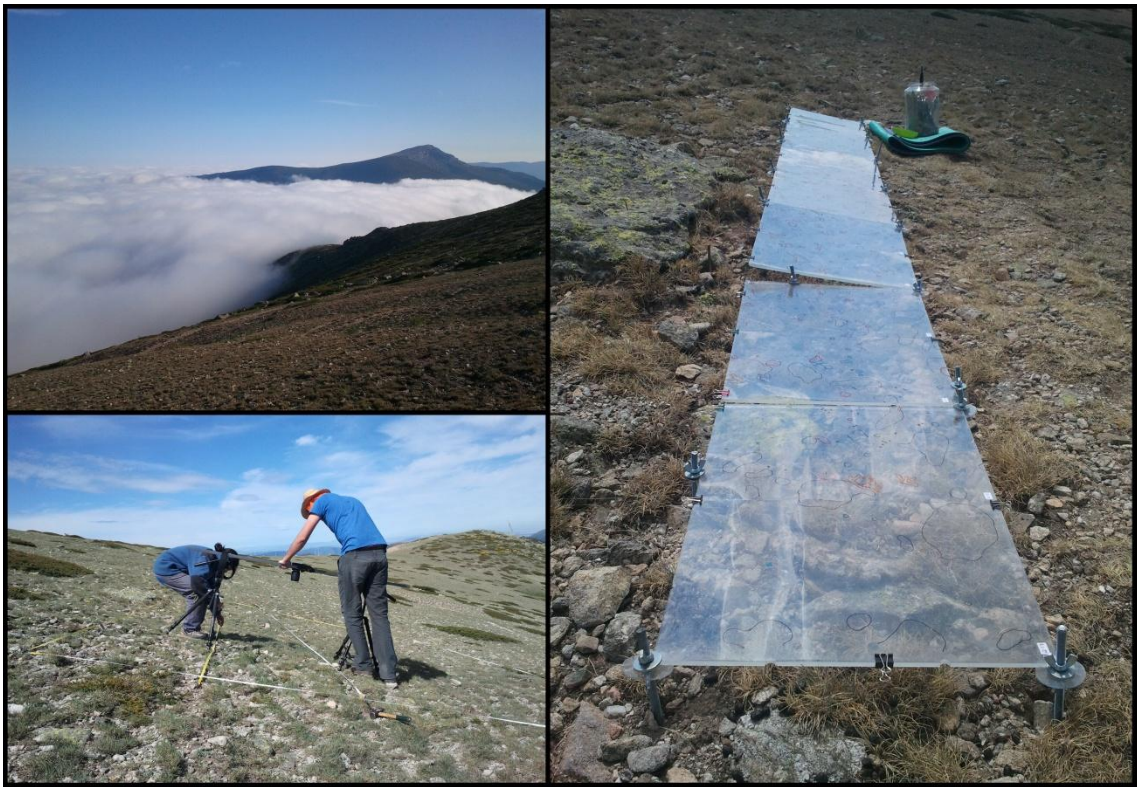
V

Maintaining distances with the engineer: patterns of coexistence in plant communities beyond the patch-bare dichotomy



Pescador, D., Chacón, J., de la Cruz, M. & Escudero, A.

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ABSTRACT

- Patched plant communities with an engineer affecting the performance and patterns of coexisting species are the norm under stressful conditions. Applying plant coexistence theory concepts to stressful communities structured in two-phase mosaics is a challenge.
- To unveil the mechanisms governing coexistence in these communities, we have developed a *new point-raster* approach of spatial pattern analysis, which was applied to a Mediterranean high mountain grassland to show how *Festuca curvifolia* patches affect the local distribution of coexisting species.
- We recorded 22,111 individuals of 17 plant perennial species. Most coexisting species were negatively associated with *F. curvifolia* clumps but at relatively short distances. Bivariate nearest-neighbor analyses confirmed this behavior, with the majority of coexisting species inhibited by *F. curvifolia* at short distances (between 0-2 cm) and up to 8 cm in some cases.
- Our study suggests the existence of a fine scale effect of *F. curvifolia* for most species promoting coexistence through a mechanism we call ***facilitation in the halo***. Most coexisting species are displaced to an inter-phase area between patches, where two opposite forces reach equilibrium: attenuated severe conditions by proximity to the island of fertility and competitive exclusion mitigated by avoiding the *F. curvifolia* canopy.

Key words: coexistence, engineer, interactions, Mediterranean high mountain, nearest-neighbor analysis, *point-raster* approach, Ripley's *K*-function, spatial heterogeneity.

INTRODUCTION

Two-phase plant community patterns with plants grouped in conspicuous patches on a bare ground matrix is the norm in harsh habitats (Wiens, 1976). These patterns can vary from stripes and arcs to spotted clumps of plants as a function of slope anisotropy (Deblauwe *et al.*, 2011). Previous studies have attributed pattern formation to abiotic processes related to water re-allocation through run-off and infiltration in arid regions (Ludwig *et al.*, 2005; Puigdefábregas, 2005), and to topography or other external agents such as soil particle size (Cox & Larson, 1993; Pérez, 2012), differential soil resource distribution (Kershaw, 1963), unidirectional wind (Okin & Gillette, 2001) or cryoturbation and solifluxion in arctic and alpine ecosystems (Liptzin, 2006). Vegetated patches in alpine ecosystems are usually dominated by one or few species with graminoid or cushion-like habits (Körner, 2003), while patches in arid regions are usually dominated by shrubs, which act as genuine ecosystem engineers affecting the ecosystem as a whole (Jones *et al.*, 1994; Badano *et al.*, 2006). Furthermore, some demographic processes of these dominant species -frequently just one- determine both the construction and the degenerative stage of the clumped structure and resulting in ´patch dynamics´(Aguiar & Sala, 1999).

The existence of two-phase structures has a great effect on critical ecosystem processes, like water dynamics and nutrient cycling, and on the direction and intensity of biotic interactions that plants establish with their neighbors (Theodose & Bowman, 1997). Vegetation clumps are the result of intrinsic self-organized mechanisms related to the performance of dominant species (Lejeune *et al.*, 1999), where feedbacks between biomass (above-below ground relationships) and water (Gilad *et al.*, 2004; Meron, 2012) can be controlled by plant-plant interactions. Namely, concepts such as *islands of fertility* (Burke *et al.*, 1998) or *nurse-plants* (Callaway & Walker, 1997) were proposed, and a new paradigm in which facilitation is prevalent in stressful habitats arose (Callaway *et al.*, 2002; Bruno *et al.*, 2003; Brooker *et al.*, 2008). The effect of patchy structures on diversity has also been explored including its functional (Michalet *et al.*, 2011) and phylogenetic components (Butterfield *et al.*, 2013) at different spatial scales (Cavieres *et al.*, 2014).

Previous studies on two-phase communities in alpine environments have generally found that the main engineers in the community exert a positive (i.e., facilitative) effect (Badano & Cavieres, 2006; but see, de Bello *et al.*, 2011; Dvorský *et al.*, 2013). Most of these studies base their conclusions on positive associations detected between plants at some predetermined spatial scale (usually the scale of the vegetated patch), which is usually interpreted to be the result of positive interactions. However, by only focusing on a predetermined scale, this approach does not detect other possible causes of positive associations, such as the existence of shared microhabitat linked to heterogeneous resource distribution (Seabloom *et al.*, 2005). Environmental heterogeneity is known to affect the spatial distribution of organisms and can confound the detection of positive and negative associations between them (Getzin *et al.*, 2008). On the other hand, association or repulsion between species can be mediated by specific attributes such as plant size (Yeaton *et al.*, 1977; Phillips & MacMahon, 1981), life-history strategies (Michalet, 2007) and functional attributes (Schöb *et al.*, 2012) which could condition the scale at which positive or negative associations occur. All this makes the use of just one spatial scale unsatisfactory if we want to gain insight on how and why diversity is structured at different scales in these communities. In other words, we can only separate the 1st and 2nd order spatial effects on realized patterns by simultaneously considering a wide array of scales using appropriate techniques.

We are witnessing an explosion of studies using spatial statistics in ecology (Wiegand & Moloney, 2004; Perry *et al.*, 2006; Law *et al.*, 2009; Wang *et al.*, 2011; Lan *et al.*, 2012). Spatial point pattern analysis can help to infer the ecological mechanisms underlying the spatial structures within the community (Mcintire & Fajardo, 2009; Brown *et al.*, 2011). These methods are especially suited for detecting the spatial scales at which ecological processes (i.e. those linked to environmental heterogeneity and plant coexistence; Getzin *et al.*, 2008; Shen *et al.*, 2013) and pure biological processes (i.e. plant interactions and dispersal ; Amarasekare, 2003; Wiegand *et al.*, 2009) are operating. A panoply of analyses are routinely used to evaluate spatial patterns and their underlying mechanisms (Wiegand *et al.*, 2013) by considering each plant as a discrete point in space (Ripley, 1981; Stoyan & Stoyan, 1994; Diggle, 2003). However, when the shape or size of the plant is relevant, a point pattern perspective may not be efficient in exploring spatial plant relationships at fine scales (Nuske *et al.*, 2009). In fact, the point assumption is only reasonable if the area of events is small relative to the spacing between them (Dixon, 2006). The only study of two-phase communities (that we know of) where these techniques have been employed is the one conducted by Fajardo *et al.* (2008). These authors recognized the misuse of employing point assumptions. However, there are other alternatives such as the so-called *raster approach* (Wiegand *et al.*, 2006), which was developed for cases where individuals are better represented by shapes than by points. In this study, we propose the use of a *point-raster approach* as an intermediate approach between the usual point pattern and *raster approach*. In this case, individuals of the dominating species forming the patch are represented by a grid of points delineating their real shape, whereas the individuals of the accompanying species are represented by single points. This is convenient as the individuals of dominant species are more than one order of magnitude larger than the other species. This allows us to use spatial point pattern techniques and solves the problem of the extensive individuals of the engineer plant.

Other neglected point pattern techniques, such as Berman's test (Berman, 1986) and point process modeling (Baddeley & Turner, 2000), can still help determine the factors conditioning the spatial distribution of species within the community and distinguish heterogeneity from pure biological processes.

With this in mind, we studied a pasture-like alpine Mediterranean community above the treeline. This type of community is a good example of a patched community in which spots and stripes are perpendicular to the slopes due to environmental severity. These patches are formed by clumps of the perennial graminoid, *Festuca curvifolia* Lag. *ex* Lange, together with more than 20 perennial specialists interspersed within the clumps or directly in the ground terraces between stripes. We mapped a well-conserved area in this community in Sierra de Guadarrama in Central Spain. We asked whether *F. curvifolia* patches affect the distribution of the other perennial species in the community and at which spatial scales this occurs. Since we expected the pattern of each perennial species in the community to be profoundly affected by the presence and abundance of this grass, we specifically tested (i) whether the occurrence of each perennial within or outside the clumps is more or less frequent than expected by chance; (ii) the existence and range of short-scale attraction or repulsion, and (iii) whether *Festuca curvifolia* is responsible for the heterogeneous distribution of the other perennial species.

MATERIALS AND METHODS

Study site and sampling

This study was conducted in a Mediterranean high mountain grassland located in the Sierra de Guadarrama National Park in the center of Spain. The climate in this region is Mediterranean with a mean annual temperature of 6.4ºC and a mean annual precipitation of 1350 mm with an intense summer drought (Navacerrada Pass weather station; 40°46' N, 4°19' W; 1860 m a.s.l.). This community extends far above the timberline, which is located between 1900-2000 m a.s.l. and is dominated by Scot pines (*Pinus sylvestris* L.) interspersed in a shrubby-pasture matrix dominated by *Cytisus oromediterraneus* Rivas Mart. *et al*. and *Juniperus communis* L. subsp. *alpina* (Suter) Čelak. The vegetation in these alpine *islands* is organized in a two-phase structure dominated by *Festuca curvifolia*. This perennial graminoid represents the main structural and cover element in this community, forming small ellipsoidal-shaped patches and stripes, which do not normally exceed 30 cm in length, in a bare ground matrix (Supporting Information, Fig. S1, Fig. S2). The community also includes several local endemisms, such as *Jasione crispa* (Pourr.) Samp., *Pilosella vahlii* (Froel.) F.W. Schultz & Sch. Bip., *Minuartia recurva* (All.) Schinz & Thell., *Armeria caespitosa* (Gómez Ortega) Boiss. in DC. or *Erysimum penyalarense* (Pau) Polatschek. together with arctic-alpine elements, such as *Agrostis rupestris* (All.), which reach their latitudinal southernmost limit here.

In the summer of 2012, we established a rectangular 9 x 6 m plot (40º47´10´´ N; 3º58´51´´ W; 2210 m a.s.l.) in a homogenous and representative area of this community. The community was fully mapped and plant sizes were recorded by drawing the outline of each perennial plant on transparent DIN-A3 sheets of *polyvinyl-chloride* (PVC), which were supported in a mobile methacrylate structure suspended 5 cm over the soil surface. In the case of clonal species, we drew the outline of each independent clump and considered each clump to be independent individuals. We performed cross-slope transects with this system throughout the entire plot area. Finally, we recorded 30 random X- Y coordinates in the plot with a GPS Leica Viva GS15 system to geo-reference the sheets mosaic with a precision below 2 cm.

Digitalization and layer compilation

A total of 420 PVC sheets were recorded and converted to digital images with a photo-scanner (Epson Expression 10000XL). All images were assembled in a unique picture with Adobe Photoshop CS3 software (Adobe Systems, San Jose, CA) and compressed to jpeg format. The image was geo-referenced to the projection UTM 30N–ETRS89 using the 30 random X-Y coordinates. Each individual plant recorded was then digitalized in ArcGIS 10.1 software (ESRI, 2011) with the help of the interactive pen display Wacom DTU-2231 (Vancouver, WA, USA). First, we obtained a vector layer for each species with a polygon for each individual. We then measured the total area, perimeter, maximum and minimum axes (length and width) and centroids (plant center) of each polygon (i.e. individual). Using the vector layer for *Festuca curvifolia,* we built two types of maps. We first built a raster map with a cell size of 5 cm representing the percentage cover of *F. curvifolia* per cell. Secondly, we built a map transforming *F. curvifolia* polygons into *masses of points*, i.e., each polygon was converted into a grid of points (separated by 0.5 cm in accordance with the smallest *F. curvifolia* clump) which accurately preserved clump size and shape. This map was created with ArcGis 10.1 software (ESRI, 2011).

Statistical Analysis

To test the role of the dominant plant species, we focused on the influence of *F. curvifolia* at two scales. The first scale was a fine scale at which the observed patterns were generated by direct plant-plant interactions. This scale was examined using the classical contingency table approach for biphasic communities which considers space as a template in which two alternative stages are possible (i.e., bare ground or *F. curvifolia* clumps). We also used a new point pattern analysis technique (distribution function of nearest neighbor distances between points and *masses of points*). The second scale was a coarse scale at which we evaluated the role of *F. curvifolia* cover as a driver of first order heterogeneity of coexisting species patterns, as well as distribution and local density of each species.

Contingency table approach

We analyzed if there was a pairwise association between *F. curvifolia* clumps and the 10 most abundant species in the community (i.e., species with more than 100 individuals; hereafter coexisting species). We tested whether the occurrence of each coexisting species in *F. curvifolia* clumps and in the bare ground was greater or lower than expected by chance. For this, we built a contingency table for each coexisting species with the observed numbers of individuals that appeared inside and outside *F. curvifolia* patches and the expected numbers under the assumption of independence (i.e., dividing the total number of individuals among the inside and outside classes proportionally to the surface area occupied by *F. curvifolia* clumps and bare soil, respectively). We computed a Fisher´s exact test for each species’ contingency table.

Small scale association between F. curvifolia clumps and the coexisting species

To evaluate the small scale association between *F. curvifolia* clumps and the coexisting species, we computed the so-called (bivariate) nearest neighbour distance function distribution [$G_{ij}(x)$; Diggle, 2003], i.e., the cumulative distribution function of the distance from each coexisting species to the nearest *F. curvifolia* individual. As *F. curvifolia* clumps are considerably larger than the individuals of coexisting species, we approximated these individuals to a point using their centroids, whereas *F. curvifolia* clumps were considered with the 0.5 cm grid of points. This *point-raster* approach, an intermediate approach between classical point pattern techniques and those of Wiegand *et al.* (2006) and Nuske *et al.* (2009) for extensive individuals, allows the accurate estimation of nearest-neighbor relationships between objects of different types (points and shapes). On the other hand, representing the accompanying species as points allows the use of the toolset of point process modeling for model fitting and inference. Besides, individual centroid usually coincide with the rooting point (i.e. the establishment point), and then we can explore the effects of patches on species establishment.

Traditionally, inference for bivariate point pattern analysis has relied on the well-known method of toroidal shift (Goreaud & Pélissier, 2003). This is inadequate when individual patterns are not stationary. Instead, we tested G_{ij} functions computing the usual Monte Carlo envelopes simulating each accompanying species from an appropriate null model i.e., a model which describes the small and large scale properties of the realized patterns (Wiegand & Moloney, 2004). To select the appropriate null model for each coexisting species, we followed an approach conceptually similar to the pattern reconstruction strategy of Wiegand *et al.* (2013).We first fitted a battery of different spatial models for each species: a) a homogeneous Poisson process with constant intensity λ equal to the density of the observed pattern; b) a homogeneous Poisson cluster process with constant intensity λ and parameters σ and ρ fitted by minimum contrast (Diggle, 2003); c) 16 heterogeneous Poisson processes with an intensity function $\lambda(x,y)$ estimated with a Gaussian kernel (Wiegand *et al.*, 2007) with 16 different σ values to experiment different bandwidths from σ = 0.5 to σ = 2.0 m; and finally, d) 16 heterogeneous Poisson cluster processes (Waagepetersen, 2007) with parameters σ and ρ fitted by minimum contrast and intensity functions estimated as in c). We then evaluated their overall fit with the goodness-of-fit *u* statistic (Diggle, 2003; Loosmore & Ford, 2006),

$$u = \int_{r=0}^{r_{max}} \left\{\widehat{K}(r) - \overline{K}(r)\right\}^2 \, dr$$

where $\widehat{K}(r)$ is the estimation of Ripley's K-function (Ripley, 1977) for each coexisting species, $\overline{K}(r)$ the mean of the K functions of 199 patterns simulated according to the evaluated null model, and *r* is the range of spatial scales at which the functions are estimated. We estimated the K functions with *r* up to 150 cm with 1 cm lags. After adjusting and evaluating all the mentioned models, we selected the model which produced the smallest *u* value as the "appropriate" model for each species. When evaluating heterogeneous models, we substituted the inhomogeneous-K function (Baddeley *et al.*, 2000) for K in the calculation of *u*.

Inferences about the association between each coexisting species and *F. curvifolia* clumps were obtained by comparing the empirical G_{ij} function to the 5th -lowest and 5th- highest values of 199 G_{ij} functions computed keeping the *F. curvifolia* grid of points "fixed" and simulating the distributions of each species' individuals obtained by the appropriate null model. For each envelope test, we computed a goodness of fit test (Diggle, 2003; Loosmore & Ford, 2006; Baddeley *et al.*, 2014) in the range where there were apparent significant differences (i.e. when the observed pattern remained outside the envelopes built under the null model).

Large scale effects driven by F. curvifolia cover on coexisting species patterns

We first analyzed the association between the spatial distribution of each coexisting species and *F. curvifolia* clumps by computing the Z1 statistic of Berman (Berman, 1986). This statistic tests the association between a point pattern and a covariate. It is defined as $Z1 = (S-\mu)/\sigma$, where *S* is the sum of the values of the covariate at each data point, and μ and σ are the mean and standard deviation of the values of the covariate in the study area, respectively. Under the null hypothesis of no association, *Z1* would have a standard normal distribution. As covariates we used a raster map with 5 cm resolution summarizing the percentage cover of *F. curvifolia* and another raster map with a 1 cm resolution with the distance from each pixel to the nearest *F. curvifolia* clump.

Finally, we explored whether *F. curvifolia* clumps had significant effects on the large scale spatial pattern (i.e., in the first order heterogeneity) of the coexisting species. For this, we fitted heterogeneous Poisson processes to each species assuming that the intensity function $\lambda(x)$ was dependent on *F. curvifolia* cover. Thus, we employed the ppm() function of *spatstat* (Baddeley & Turner, 2005) which fits the intensity of the observed point pattern as a log-linear function of one or several covariates maximizing likelihood (Baddeley & Turner, 2000). As covariates, we used several versions of the *F. curvifolia* cover map (at 1, 5, 10, 20 and 50 cm pixel resolutions).We fitted intensity surfaces to each of these *F. curvifolia* maps for each species. These surfaces were used to compute the inhomogeneous-K function (Baddeley *et al.*, 2000) and to simulate heterogeneous Poisson patterns (HPP) as a basis for testing whether the fitted intensity surfaces accounted for the heterogeneity of each species. For this, we compared the empirical inhomogeneous L-function $\left[L(r) = \sqrt{\frac{K(r)}{\pi} - r}\right]$ to the 5th -lowest and 5th-highest values of 199 simulations of the fitted HPP processes. If the inhomogeneous L-function remained inside the envelopes at large spatial scales, we concluded that the model explained the heterogeneous pattern of the tested coexisting species.

All statistical analyses were conducted in R version 3.0.2 (R Core Team, 2012) using the packages *spatstat* (Baddeley & Turner, 2005) and *ecespa* (De la Cruz, 2008).

RESULTS

We recorded a total of 22,111 individuals of 17 perennial plant species of which *F. curvifolia* was the most abundant (Table 1). The bare ground fraction occupied 67.84 % of the plot, and *F. curvifolia* dominated the vegetation fraction with a cover of 26.19 % (Table 1). *F. curvifolia* individuals had a mean size of 12.67 cm^2, with 25% of individuals being smaller than 1 cm^2 and 75% not exceeding 6.35 cm^2. The largest *F. curvifolia* individual reached 3,405 cm^2. Mean nearest neighbor distance between *F. curvifolia* individuals was 1.03 cm (±0.87), and the greatest distance was 8.48 cm.

Contingency table analyses showed that all coexisting species except *E. penyalarense* and *J. crispa* were negatively associated with *F.curvifolia* (Table 2). This suggests that most species tended to appear more frequently outside *F. curvifolia* clumps.

Table 1. Descriptive statistics of the species present in the Guadarrama plot. Total number of observed individuals, individuals inside of *F. curvifolia* clumps, mean and standard deviation of distance to *F. curvifolia* border (cm), mean and standard deviation of maximum individual diameter (cm), total area occupied and percentage of cover of each species are provided. Species in bold (more than 100 individuals in the plot) represent the coexisting species of *F. curvifolia* considerated in all statistical analyses. Nomenclature of species and subspecies was standardized according to Flora Iberica (Castroviejo 1986-2012; http://www.floraiberica.org/ [10/28/2013]).

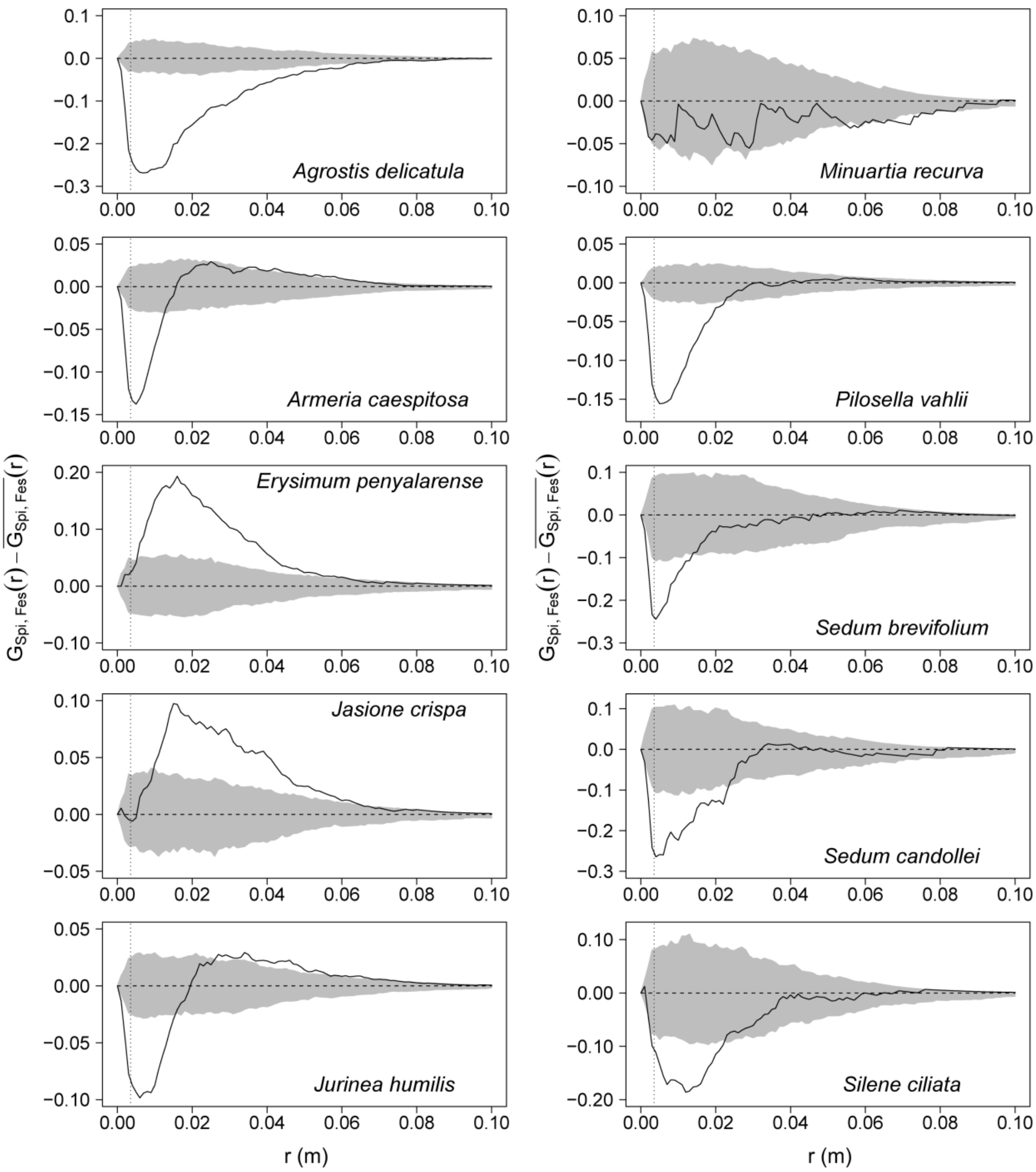
Species	Inds. obs.	Inds. obs. inside of *F.curvifolia*	Distance to *F.curvifolia* mean±s.d. (cm)	Max. ind. diameter mean±s.d. (cm)	Area total (m^2)	Cover (%)
Agrostis delicatula Pourr. ex Lapeyr.	1072	35	2.14±1.65	1.69±0.89	0.18	0.37
Armeria caespitosa (Gómez Ortega) Boiss. in DC.	2841	256	1.56±1.41	1.16±0.58	0.27	0.55
Biscutella valentina subsp. *pyrenaica* (A. Huet) Grau & Klingenberg	1	0	1.08	4.43	$7.05\cdot10^{-4}$	<0.01
Deschampsia flexuosa (L.) Trin.	13	0	1.71±0.72	3.13±2.68	$8.83\cdot10^{-3}$	0.02
Erysimum penyalarense (Pau) Polatschek.	486	140	0.92±1.22	1.07±0.43	$4.1\cdot10^{-2}$	0.08
Festuca curvifolia Lag. *ex* Large	10035	-	-	3.8±5.42	12.71	26.19
Jasione crispa (Pourr.) Samp.	906	203	1.18±1.34	4.7±2.81	0.79	1.63
Juniperus communis subsp. *alpina* (Suter) Célak	7	2	1.56±1.22	8.06±5.94	$2.29\cdot10^{-2}$	0.05
Jurinea humilis *(Desf.) DC.*	1989	317	1.47±1.37	2.51±1.3	0.47	0.97
Luzula hispanica Chrtek & Krísa	14	1	2.59±1.96	3.07±1.25	$8.12\cdot10^{-3}$	0.02
Minuartia recurva (All.) Schinz & Thell s.l.	219	36	1.73±1.84	3.68±2.38	0.19	0.39
Pilosella vahlii (Froel.) F.W. Schultz & Sch. Bip.	3568	368	1.68±1.49	1.9±0.76	0.59	1.21
Rumex acetosella L.	55	3	1.43±1.22	0.81±0.33	$1.74\cdot10^{-3}$	<0.01
Sedum brevifolium DC.	455	46	1.57±1.57	0.84±0.65	$2.34\cdot10^{-2}$	0.05
Sedum candollei Raym.-Hamet	152	5	1.78±1.52	0.74±0.19	$5.64\cdot10^{-3}$	0.01
Senecio carpetanus Boiss & Reuter	7	0	1.23±0.56	3.05±2.09	$3.03\cdot10^{-3}$	0.01
Silene ciliata Pourr.	291	39	1.89±1.59	4.02±2.49	0.29	0.60
Total	22111	1451	1.59±1.48	2.83±3.94	15.60	32.15

Table 2. Results of Fisher´s exact test and Berman´s test for each coexisting species. Fisher´s exact test results (odds ratio and *P*-value) evaluate the significance of a contingency table with expected and observed individuals of each species found inside and outside *F. curvifolia* clumps. Berman´s test performed a goodness-of-fit test of the Poisson point process model for each coexisting species' point pattern dataset. The value of each covariate (distance to *F. curvifolia* and *F. curvifolia* cover) is evaluated at the coexisting species points and its sum is compared to their expected value according to the distribution of covariate values in the whole plot. Significant results are in bold.

Species	**Fisher´s exact test**		**Berman Distance**		**Berman Cover**	
	Odds ratio	*P*-value	Z1	*P*-value	Z1	*P*-value
Agrostis delicatula	0.10	**<0.001**	11.06	**<0.001**	-7.95	**<0.001**
Armeria caespitosa	0.28	**<0.001**	3.93	**<0.001**	-9.28	**<0.001**
Erysimum **penyalarense**	1.14	0.38	-4.92	**<0.001**	4.34	**<0.001**
Jasione crispa	0.82	0.07	-3.18	**0.001**	-0.91	0.362
Jurinea humilis	0.53	**<0.001**	1.16	0.247	-0.70	0.482
Minuartia recurva	0.56	**0.02**	2.11	**0.035**	-2.19	**0.028**
Pilosella vahlii	0.32	**<0.001**	6.81	**<0.001**	-8.38	**<0.001**
Sedum brevifolium	0.32	**<0.001**	1.98	**0.048**	-0.57	0.570
Sedum candollei	0.10	**<0.001**	2.27	**0.023**	-1.27	0.206
Silene ciliata	0.44	**<0.001**	3.94	**<0.001**	-2.96	**0.003**

Bivariate nearest neighbor distance results showed that *J. crispa* and *E. penyalarense* tended to appear close to *F. curvifolia* individuals more frequently than expected (at distances between 1-6.7 cm and 0.6-6.2 cm respectively; Fig. 1). However, *F. curvifolia* exerted an inhibitory effect on most species at short distances (between 0 and 2 cm approximately) or medium distances (over 8 cm in the case of *A. delicatula* and *M. verna*). On the other hand, *A. caespitosa* and *J. humilis* showed preference to have its nearest *F. curvifolia* between 3.5-6.7 cm and 2.6-8.4 cm, respectively (Fig. 1).

Fig. 1 Bivariate nearest neighbor distance distributions from each coexisting species (Spi) to the nearest *F. curvifolia* point mass. Axis y represents the difference between the $G_{Spi,\ Fes}$ (r) observed (black line) and the mean value of 199 $G_{Spi,\ Fes}$ (r) functions computed on the same number of simulated bivariate patterns. Simulated bivariate patterns were built keeping the *F. curvifolia* point mass pattern "fixed" and simulating each species with the best adjusted model. The 5th-lowest and 5th- highest values of simulations conform the envelopes (grey area).



Berman's tests revealed that the spatial pattern of most coexisting species was significantly associated with both *F. curvifolia* cover and distance to the nearest *F. curvifolia* individual (Table 2). The sign of this association varied depending on the species and the covariate. Specifically, *E. penyalarense* and *J. crispa* were significantly and negatively associated with distance to *F. curvifolia* (Table 2). *E. penyalarense* was the only species that was positively associated with *F. curvifolia* cover, while the association between this covariate and most species was significantly negative (Table 2). It is noteworthy that only a few species were not significantly affected by *F. curvifolia* cover (e.g. *Sedum* species) or distance (e.g. *Jurinea humilis*).

All coexisting species showed heterogeneous patterns (Supporting Information Table S1). In all cases, the best models included intensity surfaces estimated with Gaussian kernels. None of the heterogeneous Poisson models fitted to *F. curvifolia* cover accounted for the heterogeneity of any of the coexisting species (Supporting Information Fig. S3).

DISCUSSION

Applying emergent coexistence paradigms (Götzenberger *et al.*, 2012; HilleRisLambers *et al.*, 2012) to two-phase plant communities dominated by a single species is a challenge for which current statistical tools are clearly inappropriate (Wiegand *et al.*, 2006). Our Mediterranean high mountain community constitutes a good example of these communities in which *F. curvifolia,* a graminoid with stripe-clonal growth, represents a genuine ecosystem engineer (Jones *et al.*, 1994). Its spatial distribution and cover (small patches close to larger ones and a cover one order of magnitude greater than all of the other species together) conforms a complex structure of patch and bare structures affecting the distribution of available resources to other species.

Our study showed that spatial pattern analyses are efficient unveiling the mechanisms of coexistence in plant communities with individuals clumped around dominant species. In a previous study in this ecosystem following a contingency table approach, Gavilán *et al.* (2002) also found that *F. curvifolia* does not appear to be associated with other species. Furthermore, in our study only two species, *E. penyalarense* and *J. crispa*, showed no preference for either of these two habitats (bare ground or graminoid clumps), and the other coexisting species had a negative association. The results for most species were concurrent with those from our Berman´s test analyses. However, the spatial structure of the two "indifferent" species had a negative value of Z1 for distance to *F. curvifolia* patches. This means that they appeared closer to graminoid clumps than would be expected by chance. *E. penyalarense* was also positively associated with areas of high *F. curvifolia* cover. In other words, when considering the spatial organization of the community, they may not be so indifferent. Most of the remaining species showed a significant positive association with distance to

F. curvifolia, and were negatively associated with *F. curvifolia* cover, suggesting competitive exclusion. The fact that most species prefer bare ground does not support the widely-accepted prevalence of facilitation in high mountain ecosystems under severe environmental conditions (Callaway *et al.*, 2002; Cavieres *et al.*, 2006; Kikvidze *et al.*, 2006; Brooker *et al.*, 2008). Nevertheless, the balance between facilitation and competition in stressed environments seems to be a dynamic process (Callaway & Walker, 1997) with interactions varying as function of shifting abiotic factors in space-time (Holmgren *et al.*, 1997), life-stages of the protagonists (Callaway, 1995; Soliveres *et al.*, 2010) or shifts induced by dynamic and spatial organization (Bolker *et al.*, 2003; Meron, 2012). However, none of these mechanisms seem to explain our findings.

The analyses of the distribution of crossed nearest-neighbor distances provide insight on the processes that may be structuring this community. For most coexisting species, *F. curvifolia* patches seem to produce an exclusion pattern at very short distances (from 0 to 2 cm), except in the case of *A. delicatula* where this distance is over 8 cm (Fig. 1). For other species, *F. curvifolia* patches seem to exert attraction at short and medium distances (i.e. *E. penyalarense, J. crispa, A. caespitosa* and *J. humilis*). The exclusion pattern at short distances suggests that our engineer species produces a repulsion halo adjacent to their clump borders. Previous works have reported the presence of this competition halo around *Hieracium pilosella*, (Boswell & Espie, 1998) and in *Stipa tenacissima* tussocks (Armas & Pugnaire, 2011). In these two cases, competition for water and some shifts in soil properties from within to around the plants are the responsible mechanism. Although water does not seem to be a limiting resource in alpine ecosystems (but see, de Bello *et al.*, 2011; Dvorský *et al.*, 2013), summer drought stress is a proven fact in Mediterranean high mountain (Cavieres *et al.*, 2006;

Giménez-Benavides *et al.*, 2007; Schöb *et al.*, 2013). This limitation is probably exacerbated in the thin, sandy-textured soils where our *F. curvifolia* grasslands occur.

Despite the conspicuous patch-bare dichotomy, the robust associations between *F. curvifolia* and most coexisting species at this halo indicate that the influence of *F. curvifolia* clumps on the distribution of the species in the community extends beyond their canopy. The realized patterns of most species being negatively associated with *F. curvifolia,* but confined to short and medium distances around its clumps, suggests the existence of a sort of *variable facilitation signature*, which abruptly turns to competition as the distance from *F. curvifolia* clumps decreases**.** This may be due to the behavior of *F. curvifolia* roots which may act as a "nutrient pump" (Richards & Caldwell, 1987), capturing soil resources. Furthermore, the aboveground biomass of *F. curvifolia* may act as a *carpet,* trapping both nutrients and water from overflow, precipitation and/or wind (Coppinger *et al.*, 1991; Ehrenfeld *et al.*, 2005). This mechanism, referred to as an "island of fertility", ameliorates conditions within patches and in adjacent areas. Previous works carried out in several localities of Sierra de Guadarrama (Escudero *et al.*, 2004; D.S Pescador & A. Escudero, unpublished) showed that soils below *F. curvifolia* patches are richer in nutrients than soils below bare ground (e.g. 4.5±1.8 mg of total N/g soil below *F. curvifolia versus* 3.7±1.4 mg of total N/g bare ground soil or 5.9±2.4% of organic carbon in soil below *F. curvifolia versus* 4.3±1.6% of organic carbon in bare ground soils). *F. curvifolia* is also scarcely palatable for sheep, cows or goats due to its foliar toughness and acicularity, providing secure anti-grazing sites (Gavilán *et al.*, 2002). The positive effects exerted by *F. curvifolia* and its resource island may become negative in the close proximity of *F. curvifolia* and within clumps because of *F. curvifolia*'s dense root system and above-ground biomass. This suggests that *F. curvifolia* competes for resources (i.e. water, nutrient and light) more efficiently than the coexisting species in the community. In summary, the facilitative effect is mediated by the strong competitive ability of the engineer (Fig. 2). Its net effect would equal the balance between positive-negative interactions and above-belowground biomass. Plants benefit from the resources accumulated under *F. curvifolia* but avoid direct competition with its canopy. Consequently, most coexisting species find themselves displaced to a *security ring* or inter-phase habitat around clumps, where environmental conditions are not as stressful as in the bare zone or as competitive as within *F. curvifolia* patches. This displacement is especially apparent in the case of *A. delicatula*, a graminoid relative of *F. curvifolia.* These two species shares many functional, especially ecophysiological, traits, which could make competition more intense (Cavender-Bares *et al.*, 2004; Valiente-Banuet & Verdú, 2008). To support these findings, we examined the relationship between the individual size (i.e. maximum individual diameter) of each coexisting species and distance to the nearest *F. curvifolia* individual. Several coexisting species (i.e., *A. caespitosa*, *J. crispa*, *J. humilis* and *P. vahlii*) had a significant negative relationship (Supporting Information Table S2), suggesting a prevalence of this mechanism at short distances. In case of *A. delicatula*, we detected the opposite pattern (Supporting Information Table S2), which is compatible with the existence of competitive exclusion.

Some species, such as *E. penyalarense* and *J. crispa*, do not seem to be affected by competition with *F. curvifolia* canopies probably because functional differences are greater, suggesting niche complementarity. *E. penyalarense* is a perennial species with deep axonomorph roots and woody-erectus stems with a maximum mean height over twice that of *F. curvifolia* stems (75.3±29 mm and 32.4±9.7 mm, respectively). *J. crispa* is a highly plastic and polymorphic species, loosely caespitose, with profusely branched stems and a prostrated-creeping growth. This allows individuals to find their way to available gaps in the clumps.

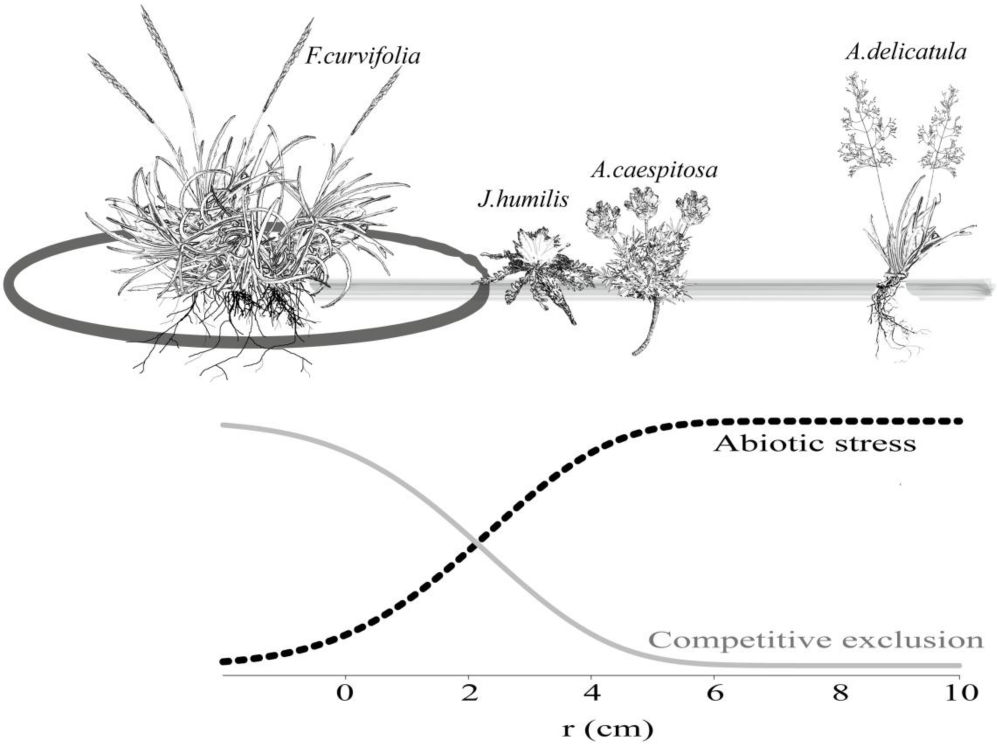


Fig. 2 ***Facilitation in the halo*** mechanism: a hypothesis for the spatial signal found at short and medium distances around *F. curvifolia*. Some coexisting species tend to occur close to the *F. curvifolia* halo where two opposite forces reach equilibrium: competitive exclusion by the *F. curvifolia* canopy (grey line) *versus* environmental severity of bare ground areas (black dotted line).

Interestingly, *F. curvifolia* cover (Supporting Information Fig. S3) did not explain the first order heterogeneity in the distribution of the coexisting species (see results of the inhomogeneous Poisson processes). This heterogeneity may be related to some soil or microclimatic gradients or to some periglaciar processes involving snow cover and duration which would affect the density and distribution of the coexisting species. Once these filters determine their coarse scale distribution in the community and *F. curvifolia* patches control their fine scale distribution. This also implies that the engineer and the coexisting species did not share the same microhabitat.

In contrast to the classical species to patch association approach, where species are only considered to be inside or outside the patch of a focal species, spatial pattern analyses of a complete community allow these associations to be described considering the entire community in a more precise context. Our methodological approach and results show how a single species may act as an ecosystem engineer that influences the distribution and abundance of the coexisting species in the community. Furthermore, the range and the sign of this influence are mediated by the morphological and functional attributes of each species. *F. curvifolia* affected the realized patterns of the majority of the species in the community at small scales, promoting what we called the ***facilitation in the halo*** mechanism. This mechanism induced a detectable spatial signature in all of the coexisting species except two which were clearly facilitated. These plants tend to occur in an inter-phase zone where two opposite forces reach equilibrium: attenuated severe conditions due to proximity to an island of fertility and competitive exclusion mitigated by avoiding the *F. curvifolia* canopy. Nevertheless, this assembly process did not explain the large scale patterns of coexisting species, since the heterogeneity imposed by *F. curvifolia* at medium or large scales was not determinant.

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SUPPLEMENTARY MATERIAL

Fig. S1. Mediterranean High Mountain pasture in our Guadarrama plot, Madrid (Spain), showing two phase structure dominated by small ellipsoidal-shaped patches and stripes of *F. curvifolia* interspersed in a bare ground matrix. Photo: A. Escudero

Table S1. Best spatial model for each coexisting species. Models tested were Poisson cluster process (PC), heterogeneous Poisson (HP) and heterogeneous Poisson cluster (HPC). The best model was selected according to the lowest non-significant goodness-of-fit statistic *u* (GOF) value. Bandwidth: σ value of the Gaussian kernel used to estimate the intensity surface. σ and ρ: fitted parameters for the heterogeneous Poisson cluster models.

Species	Model	Bandwith (m)	*u*	*P*-value	Σ	ρ
Agrostis delicatula	HPC	1.4	1.10	0.83	0.026	2.47
Armeria caespitosa	HPC	1.2	1.09	0.76	0.012	3.50
Erysimum penyalarense	HPC	1.5	0.94	0.76	0.004	8.28
Jasione crispa	HPC	1.5	0.07	0.97	0.072	4.56
Jurinea humilis	HPC	1.1	1.11	0.46	0.001	17.16
Minuartia recurva	HP	0.7	56.94	0.51	-	-
Pilosella vahlii	HPC	1.3	0.57	0.75	0.003	8.11
Sedum brevifolium	HPC	1.1	67.99	0.47	0.001	3.39
Sedum candollei	HPC	2	10.06	0.74	0.001	2.42
Silene ciliata	HPC	1.5	33.42	0.71	0.045	0.63

Table S2. Statistics of a fitted lineal model between the distance of each individual at the nearest *F. curvifolia* patch and size of individuals for each coexisting species. Adjusted coefficient of determination (R^2), the estimated coefficient (Slope) and *P*-value of a t-test for the hypothesis H_0: Slope=0. Significant results are in bold.

Species	R^2	Slope	*P*-value
Agrostis delicatula	**$9.32 \cdot 10^{-03}$**	**0.18**	**0.001**
Armeria caespitosa	**$7.89 \cdot 10^{-03}$**	**-0.22**	**<0.001**
Erysimum penyalarense	$4.92 \cdot 10^{-07}$	0.00	0.99
Jasione crispa	**$6.99 \cdot 10^{-02}$**	**-0.13**	**<0.001**
Jurinea humilis	**$3.31 \cdot 10^{-03}$**	-0.06	0.01
Minuartia recurva	$1.01 \cdot 10^{-02}$	-0.08	0.14
Pilosella vahlii	**$6.00 \cdot 10^{-03}$**	**-0.15**	**<0.001**
Sedum brevifolium	$1.45 \cdot 10^{-03}$	-0.09	0.42
Sedum candollei	$6.55 \cdot 10^{-03}$	-0.64	0.32
Silene ciliata	$1.06 \cdot 10^{-02}$	0.07	0.08

Fig. S2 Mark-correlation function of *F. curvifolia* point pattern considered individual size as a mark. The observed Kmm(r) (black line) is compared to Kmm(r) of 199 simulations computed with a random labeling null model. The 5th -lowest and 5th-highest values of simulations conform the envelope (grey area).

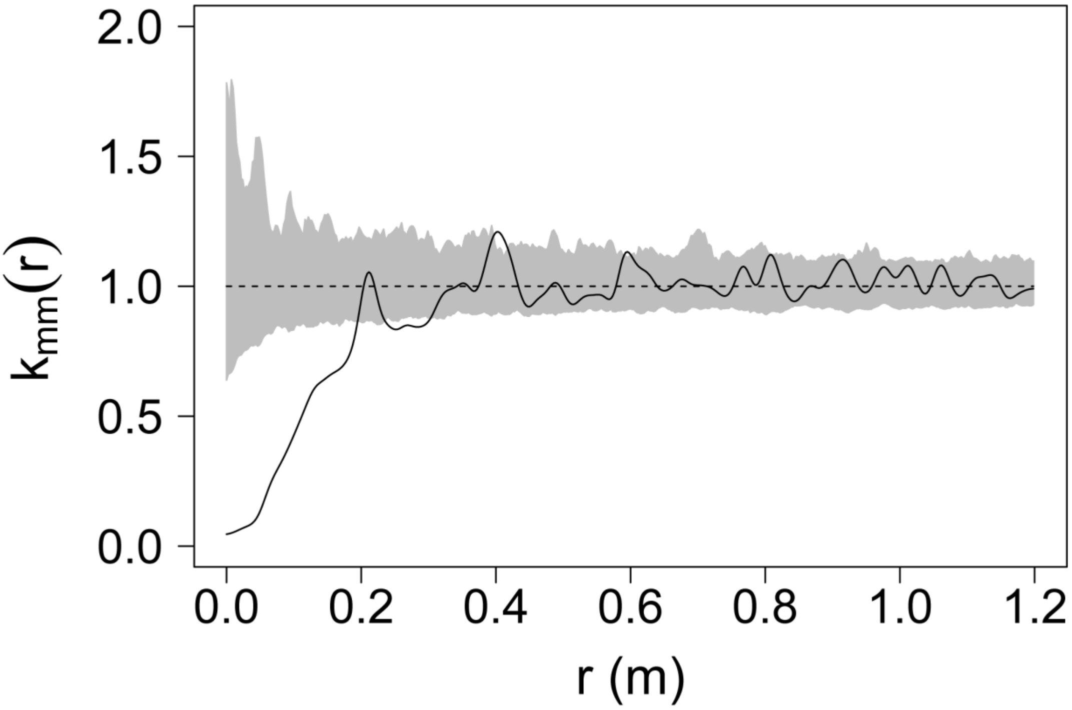
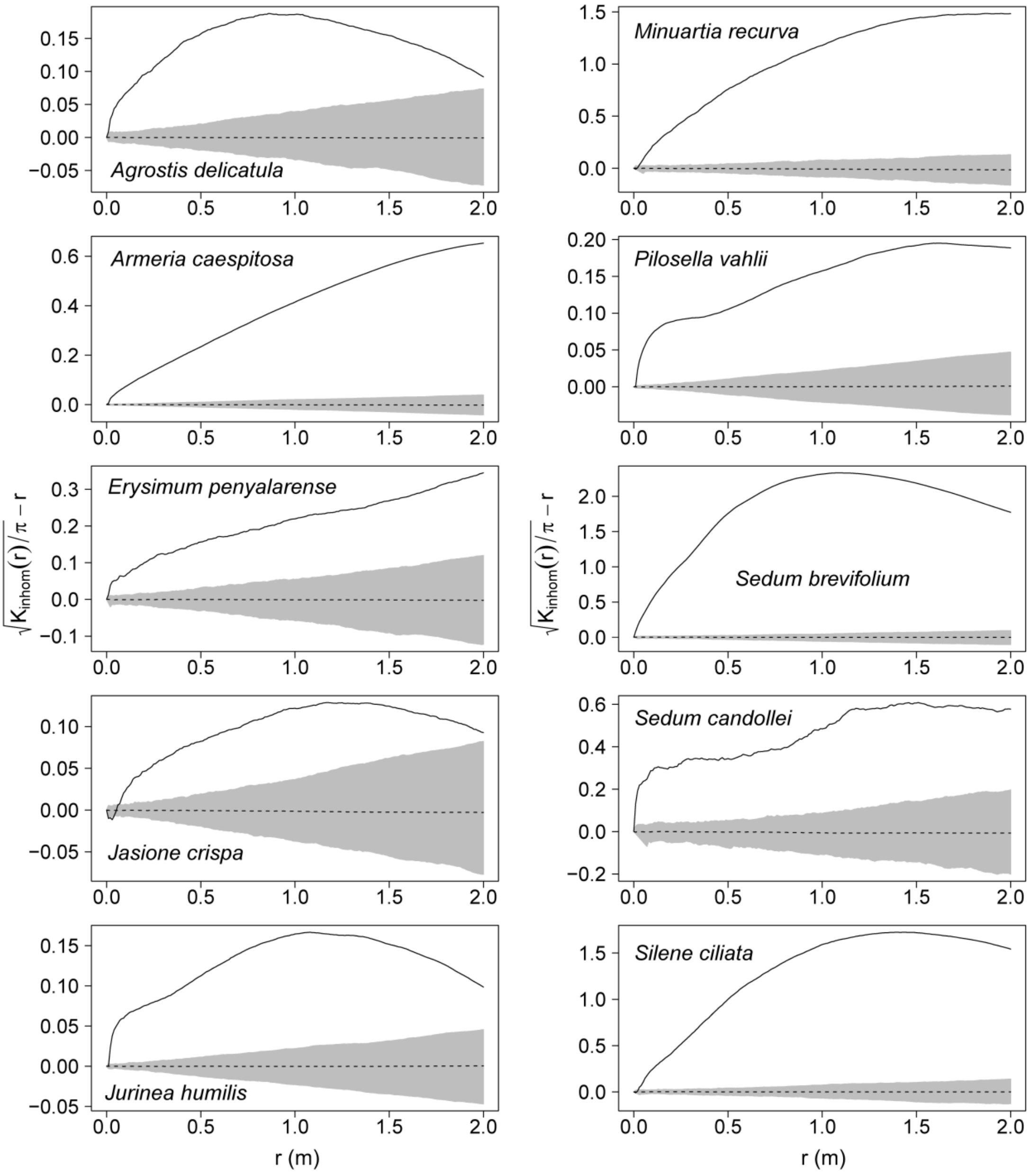


Fig. S3 Inhomogeneous L- functions of each coexisting species estimated with the intensity surfaces adjusted to the *F. curvifolia* cover map (resolution = 5 cm). The observed L-function (black line) of each species is compared to L-functions of 199 heterogeneous Poisson processes simulated from the same intensity surface. The 5th - lowest and 5th- highest values of simulations conform the envelope (grey area).



CONCLUSIONES GENERALES

- Los eventos de congelación durante la época de crecimiento fueron uno de los principales filtros abióticos relevante en el ensamble de las comunidades vegetales de alta montaña Mediterránea.
- La sequía estival característica de estos ambientes representó un concomitante filtro que podría explicar la mayor resistencia a la congelación mostrada por las especies que habitan en las montañas secas.
- La respuesta funcional de las especies de alta montaña Mediterránea sometidas a un gradiente altitudinal fue débil independientemente de considerar el nivel inter- o intra-específico.
- Los principales recursos de variación funcional se hallaron dentro de cada localidad, sugiriendo una mayor importancia del componente micro-ambiental en relación a la altitud.
- Los mecanismos de ensamblaje de la comunidad de alta montaña Mediterránea fueron espacio-dependientes, dominando la convergencia a escalas más amplias y la divergencia a escalas más finas.
- La importancia de la segregación de nicho a escalas finas moduló el filtrado ambiental a escalas más amplias.
- El anidamiento y no la modularidad fue el patrón dominante cuando los parches de vegetación presentes en la comunidad de altas montaña Mediterránea fueron descritos como redes de interacción.

- No obstante anidamiento y modularidad estuvieron correlacionados y fueron dependientes de la diversidad local además de variables estructurales como el tamaño de los parches o del pastizal.
- *Festuca curvifolia*, la especie dominante en las comunidades de pastos de la Sierra de Guadarrama, indujo una marca espacial detectable que determinó la distribución de las especies vegetales que coexisten en sus inmediaciones.
- *F. curvifolia* promovió un mecanismo que hemos denominado facilitación en el halo, mediante el cual el resto de las especies vegetales son desplazadas a una zona de inter-fase donde la severidad ambiental y la exclusión competitiva alcanzan un equilibrio motivado por *F. curvifolia*.