

Spatio-temporal variability in ecophysiological determinants of performance: a study in perennial herbs of coastal foredunes



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DEPARTAMENTO DE BIOLOXÍA CELULAR
E ECOLOXÍA

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ecophysiological determinants
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DEPARTAMENTO DE BIOLOGÍA CELULAR
E ECOLOGÍA

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COMPOSTELA,**

INFORMA:

Que la presente memoria titulada **“Spatio-temporal variability in ecophysiological determinants of performance: a study in perennial herbs of coastal foredunes”** presentada por **D. Raimundo Bermúdez Villanueva** para optar al Grado de Doctor en Biología, fue realizada bajo mi dirección en el Área de Ecología del Departamento de Biología Celular y Ecología de la Universidad de Santiago de Compostela.

Y considerando que representa trabajo de Tesis Doctoral, autorizo su presentación ante el Tribunal correspondiente.

Y para que así conste, firmo la presente en Santiago de Compostela a 16 de Enero de 2013.

Visto y place del Director,

El Doctorando,

Fdo. Dr. Rubén Retuerto Franco

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Introducción general

Los ecosistemas dunares costeros se desarrollan a lo largo de los bordes donde confluyen las tres principales masas de la superficie terrestre: la masa oceánica, la terrestre y la atmosférica. En esa intersección, donde las condiciones topográficas lo permiten, la acción combinada del viento y del mar genera los acúmulos de sedimento arenoso necesarios para la formación de las dunas (Fig. 1).

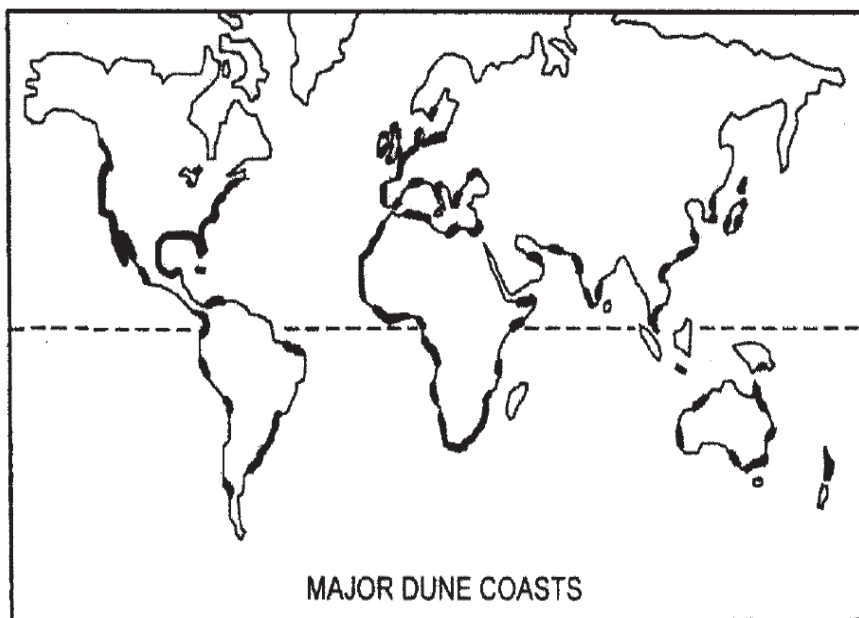


Fig. 1. Distribución de los principales sistemas dunares costeros a nivel mundial. McLachlan (2006).

Cuando sobre estos acúmulos se establecen un conjunto de especies vegetales especialmente adaptadas a la vida en estos ambientes inhóspitos, se forman los ecosistemas dunares costeros. Al igual que la acción del mar y el viento, la vegetación dunar actúa también como un factor más de formación, ya que mediante su crecimiento, las plantas contribuyen a la estabilización y fijación de las masas de sedimento. Esa estabilización permite que se vayan formando los primeros cordones dunares, paralelos a la línea de costa,

facilitando así su colonización por nuevas especies vegetales menos tolerantes, que contribuyen a que vayan aumentando en complejidad y tamaño.

Uno de los principales rasgos que caracteriza a estos ecosistemas es la gran dureza ambiental derivada de los propios factores abióticos responsables de la formación de estos ambientes (Martínez *et al.*, 2002). Sin embargo, también influyen otros factores externos como son la radiación solar, la precipitación, la temperatura, etc. La combinación de los efectos de estos factores con la acción del viento y del océano, en forma de corrientes, mareas y oleaje, sobre el sustrato arenoso, da lugar a los diferentes tipos de estrés y perturbaciones que condicionan a este tipo de ambientes. La influencia oceánica, principalmente, y del viento, disminuyen al aumentar la distancia respecto a la línea de costa. Esto da lugar a la distribución en gradientes costa-interior de los factores de estrés derivados (Rozema *et al.*, 1985; Hesp, 1991; Ishikawa *et al.*, 1995; Gustafsson *et al.*, 1996; Lortie *et al.*, 2007) y una distribución de la vegetación (Wilson *et al.*, 1999; Maun *et al.*, 1999; Dech *et al.*, 2005; Gilbert *et al.*, 2008), que se agrupa en cordones paralelos a la línea de costa, distribuidos de acuerdo a la tolerancia de las especies. Debido a la mayor intensidad que adquieren la mayoría de los principales factores de estrés cerca del océano (Fig. 2), se considera a la zona del frente dunar como el área de mayor dureza ambiental y más restrictiva para el establecimiento y desarrollo de la vegetación (Hesp, 1991; Khan *et al.*, 1998; Gagne *et al.*, 2002; Martínez *et al.*, 2002). La mayoría de los autores coinciden en destacar como principales factores de estrés a la alta salinidad y la elevada movilidad del sustrato (Maun, 1998; Wilson and Sykes, 1999; Maun and Perumal, 1999; Cheplick *et al.*, 1999; Martínez *et al.*, 2002; Dech and Maun, 2005; Gilbert *et al.*, 2008), aunque existe una gran variedad que se podrían resumir en:

Exceso de salinidad: La sal afecta a la vegetación principalmente de dos maneras: como deposición sobre las superficies aéreas de las plantas en forma de aerosol y como disolución salina del suelo a través de las estructuras subterráneas de la planta (Hesp, 1991; Maun, 1994; Cheplick and Demetri,

1999; Gagne and Houle, 2002). Los aerosoles marinos provienen de la atomización de pequeñas gotas de agua que entran en suspensión en la atmósfera y son desplazadas hacia la duna por los vientos costeros, pudiendo alcanzar importantes distancias especialmente durante las tormentas (Maun, 2009; Miller *et al.*, 2010). La concentración salina de la disolución del suelo, tiene su origen principalmente en el lavado vertical de la deposición salina de la superficie del suelo y la vegetación. En los lugares más próximos al océano, puede proceder también de incursiones marinas sobre la duna (Maun, 2009), o infiltraciones de agua marina a nivel freático (Greaver *et al.*, 2007). Los

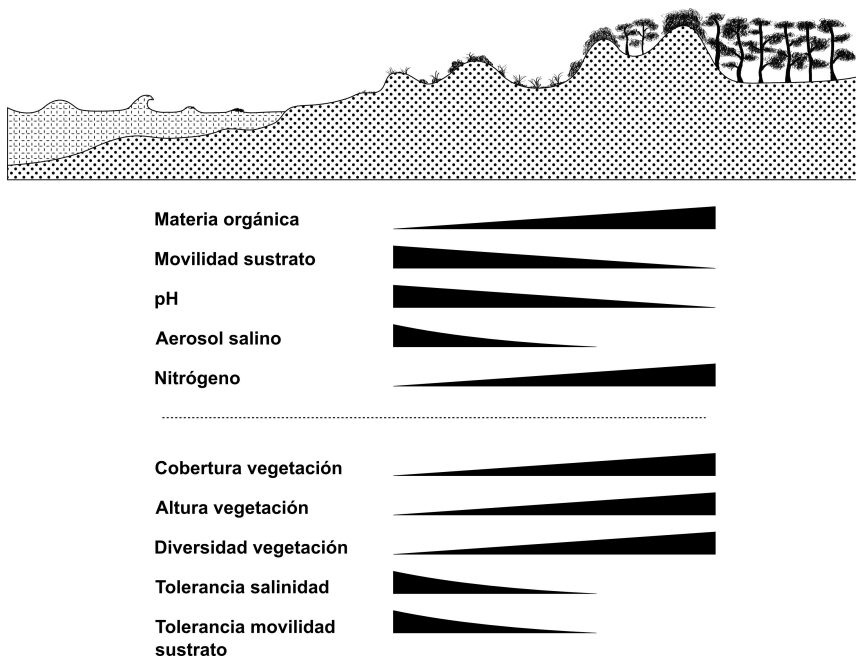


Fig. 2. Distribución a lo largo de gradientes costa-interior de los principales factores de estrés abióticos y recursos limitantes en los ambientes dunares. Basado en McLachlan (2006).

efectos de la salinidad sobre las plantas son principalmente de tipo iónico, afectando tanto a su potencial osmótico como hídrico (Khan and Aziz, 1998; Khan *et al.*, 1999; Suárez, 2011). Esto contribuye a dificultar aún más la

adquisición de agua (Suárez, 2011), y por tanto de nutrientes, a través de las raíces (Kosova *et al.*, 2011) alterando los procesos fotosintéticos (Sánchez-Blanco *et al.*, 2003; Kosova *et al.*, 2011; Suárez, 2011) y el crecimiento (Ishikawa *et al.*, 2000; Suárez, 2011). A nivel de las semillas también se ve dificultada la germinación (Maun, 2009).

Movilidad del sustrato: El sustrato tiene una textura arenosa, con predominio de partículas silíceas y calcáreas procedentes de fragmentos de conchas. Su baja estabilidad le confiere una alta movilidad, ya sea por transporte eólico o, en las zonas más próximas a la costa, a través del agua debido a la acción conjunta del oleaje, las mareas y/o las corrientes (McLachlan *et al.*, 2006). La movilidad del sustrato afecta a la vegetación de distintos modos: por un lado mediante el enterramiento, que actúa incrementando la humedad, el contenido en nutrientes y la compactación del suelo, y disminuyendo la temperatura, aireación, intensidad de luz y competencia (Maun, 1994; Maun, 1998). También dificulta la germinación de las semillas, pudiendo llegar a impedirla (Zhu *et al.*, 2009). El enterramiento de las estructuras aéreas limita o impide la función fotosintética (Maun, 1998), generando un incremento en la proporción de los costes respiratorios (Gilbert *et al.*, 2008). Por otro lado, el desenterramiento expone las estructuras subterráneas, alterando su funcionamiento o escindiéndolas (Gagne and Houle, 2002; Maun, 2009). Afecta a la captación de recursos como agua y nutrientes, a la vez que reduce la capacidad de sostén de las plantas. A su vez, la acción del transporte de partículas de arena por el viento, también actúa ejerciendo un efecto abrasivo sobre la superficie de las plantas y sus yemas, contribuyendo a la pérdida de agua y facilitando la entrada de patógenos (Yura *et al.*, 2006; Ogura *et al.*, 2008; Maun, 2009).

Contenido hídrico del suelo: La baja disponibilidad hídrica de los ecosistemas dunares terrestres está relacionada principalmente con la baja capacidad de retención característica del sustrato arenoso (de Jong, 1979). Las principales fuentes de agua para las plantas de estos ambientes tienen un

origen freático y/o atmosférico, bien sea en forma de lluvia, niebla o rocío (Barbour *et al.*, 1985; Hesp, 1991; Alessio *et al.*, 2004). El agua de la precipitación atmosférica tiene un bajo tiempo de disponibilidad en el suelo para las plantas debido a la baja capacidad de retención del sustrato, pasando rápidamente a formar parte del agua freática. En las zonas más próximas a la costa existe una marcada influencia marítima debido al agua de origen oceánico, bien sea por infiltración y mezcla a nivel freático, o por inundación debido a la acción de mareas altas y oleaje (Sternberg *et al.*, 1987; Greaver and Sternberg, 2007).

Contenido en nutrientes: Debido a la naturaleza del sustrato, formado principalmente por resistatos de silicio y fragmentos de conchas (Brown *et al.*, 2002; Franks, 2003b), es característico de estos ambientes un escaso contenido de nutrientes. Los nutrientes suelen tener un origen externo al sistema, siendo los aerosoles marinos una de las principales fuentes. Algunos iones se encuentran en alta concentración en el suelo, como Na^+ , Ca^{+2} , K^+ y Cl^- , pudiendo llegar a resultar tóxicos para las plantas (Boyce, 1954; Rozema *et al.*, 1985). Por lo general el contenido de nutrientes (macronutrientes: N, P y K) en el suelo es bajo, limitando el crecimiento de las plantas (Kachi *et al.*, 1983; Maun, 1994). Debido a su origen, normalmente tienen una distribución en gradiente costa-interior, en este caso aumentando a medida que nos alejamos de la costa (Rajaniemi *et al.*, 2009). Sin embargo, también se puede producir un pico de nutrientes de origen marino en la parte más próxima a la costa, en la duna embrionaria, debido a las arribazones de material detrítico arrastrado por las mareas (Orr *et al.*, 2005).

Radiación: La radiación incidente sobre la superficie terrestre alcanza mayores intensidades cuanto menores sean la pendiente del terreno y la cobertura vegetal. Ambas propiedades son típicas de ecosistemas dunares costeros, especialmente en las zonas más próximas a la costa (Maun, 2009), lo que contribuye a que se alcancen valores de radiación fotosintéticamente activa muy altos, llegando a alcanzar flujos de alrededor de 1800 μmoles de

fotones $\cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Maun, 2009). En estos ambientes, una cantidad importante de radiación que incide sobre la superficie del suelo, llega reflejada a la parte inferior de las hojas debido al alto índice de reflexión (albedo) característico del sustrato (Maun, 2009).

Temperatura: La elevada temperatura que puede llegar a alcanzarse en los ecosistemas dunares costeros está especialmente ligada a la alta radiación y escasa cobertura vegetal características de estos ambientes. En los momentos de mayor insolación, la temperatura superficial de suelos y plantas puede alcanzar hasta los 60°C en la superficie del suelo, descendiendo rápidamente a los pocos centímetros de profundidad (Maun, 2009). Las elevadas temperaturas tienen efectos perjudiciales para la vegetación relacionados con la pérdida de agua, a la vez que alteran los procesos fotosintéticos (Gratani *et al.*, 2009; Rubio-Casal *et al.*, 2010). En los casos más extremos pueden llegar a producirse incluso daños en la estructura de las proteínas y su desnaturalización (Kosova *et al.*, 2011). También puede ocurrir el proceso contrario, alcanzándose bajas temperaturas, llegando incluso a producirse heladas (Rubio-Casal *et al.*, 2010).

Además de los factores de tipo abiótico, en las dunas costeras también actúan factores bióticos de cierta relevancia. Éstos suelen ocurrir por interacciones entre especies (o por distintos individuos dentro de la misma especie), siendo el principal la competencia. Este proceso también ocurre a lo largo de un gradiente costa-interior, pero en esta ocasión suele mostrar el mayor grado de restricción hacia el interior donde se incrementa la densidad de vegetación (McLachlan and Brown, 2006; Martínez *et al.*, 2008; Maun, 2009), aunque con excepciones como el caso de algunas especies como *Ammophila arenaria* u otras herbáceas colonizadoras (Martínez and García-Franco, 2008). Además de los procesos de competencia, existen otras interacciones bióticas importantes que pueden tener efectos positivos, como ocurre con el establecimiento de relaciones micorríticas (Hesp, 1991; Cakan *et al.*, 2006; Camprubi *et al.*, 2010; Camprubi *et al.*, 2011a) al producirse una

asociación a nivel de la rizosfera entre un hongo y una planta favoreciendo la adquisición de nutrientes, o con la facilitación, en la que la presencia de una planta favorece el establecimiento y crecimiento de otra (Gagne *et al.*, 2001; Rudgers *et al.*, 2003; Franks, 2003a; Armas *et al.*, 2009).

Además del gran número y tipo de factores de estrés y perturbaciones que afectan a las plantas, así como las interacciones que entre ellos ocurren, los ecosistemas dunares costeros tienen otras peculiaridades derivadas de su naturaleza (viento, olas, etc.) que le confieren si cabe un aún mayor grado de dureza. Entre ellas destacan su gran variabilidad en intensidad y frecuencia, llegando a alcanzar grandes amplitudes de variación (Hesp, 1991; Wilson and Sykes, 1999; Dech *et al.*, 2006; Maun, 2009; Rubio-Casal *et al.*, 2010; Reed *et al.*, 2010). Pueden lograr, en cortos periodos de tiempo, intensidades muy altas o muy bajas para un mismo factor de estrés, como vimos en el ejemplo de las temperaturas superficiales de suelo y planta (Rubio-Casal *et al.*, 2010). Otra fuente de variación a tener en cuenta es la frecuencia con la que se repite un estrés o perturbación (Maun, 1998; Gagne and Houle, 2002; Dech and Maun, 2006), pudiendo ser desde muy recurrentes a poco frecuentes. Muy relacionado con la frecuencia, está la predecibilidad (Maun, 1998; Alpert *et al.*, 2002; Orr *et al.*, 2005; Maun, 2009; Reed *et al.*, 2010), siendo la aparición de esos factores de estrés y perturbaciones en muchos casos difícilmente predecibles para la planta. La impredecibilidad dificulta que las especies puedan desarrollar una respuesta tan efectiva como ocurriría en ambientes más estables, por lo que, debido a esto, las especies tienden a adquirir adaptaciones a las condiciones intermedias (Alpert and Simms, 2002; Karban *et al.*, 2004). De este modo se ve reducido el rango de posibles respuestas que la planta puede desarrollar, viéndose reflejado en una disminución de la productividad (Alpert and Simms, 2002; Volis *et al.*, 2002). Como resultado, a la planta le resulta más complicado poder anticiparse e ir desarrollando la respuesta más adecuada. Estos efectos se ven agravados cuando, además, los factores de estrés y las perturbaciones inciden de forma prácticamente instantánea, al

tener la planta un menor tiempo de reacción que cuando sucede de un modo más progresivo. Se impide o limita así la actuación desde el inicio de algunos mecanismos de respuesta que requieren mayor cantidad de tiempo para desarrollarse.

ADAPTACIONES

Para superar estas restricciones, las especies que habitan estos ecosistemas desarrollaron una serie de adaptaciones que le permiten dar respuesta desde a cambios instantáneos y puntuales, hasta a más progresivos y continuados. Algunos ejemplos de los principales mecanismos desarrollados por las diferentes especies son:

Estructuras subterráneas bien desarrolladas: Especialmente las especies del frente dunar muestran estructuras de captación y almacenamiento del tipo de raíces, rizomas y/o bulbos, bien desarrollados en profundidad y estructura (Maun, 1994; García-Mora *et al.*, 1999; Ripley *et al.*, 2004; Maun, 2009; Suárez, 2011). Estas adaptaciones van dirigidas a incrementar el volumen de suelo explotado, por parte de las raíces de estas plantas, para la obtención de recursos, a la vez que para el almacenamiento de éstos en otras estructuras como rizomas y bulbos. Contribuyen así a que la planta pueda mantener el suministro de recursos y tolerar episodios de escasez o dificultad para su adquisición.

Tolerancia al enterramiento: Las principales estrategias seguidas por las especies ante el enterramiento, conducen hacia un incremento en la velocidad de crecimiento, especialmente de la parte de la planta que se ve afectada (biomasa aérea). Se produce principalmente mediante la elongación de los meristemos apicales y entrenudos, y/o el incremento en el número de nudos (Disraeli, 1984; Maun, 1998; Maun, 2009), para compensar la superficie foliar (fotosintetizadora) perdida. A su vez también se produce la movilización de los recursos contenidos en las estructuras enterradas, hacia la producción de

nuevas estructuras (Gilbert and Ripley, 2008; Gilbert *et al.*, 2008; Maun, 2009), minimizando por otro lado los gastos respiratorios que requeriría el mantenimiento de las antiguas estructuras.

Esclerofilia: La adquisición de una cutícula gruesa confiere a la planta la capacidad de tolerar mayores intensidades de algunos de los factores de estrés que ocurren en las dunas costeras, especialmente en el frente dunar, como son el efecto del viento y la abrasión (Hesp, 1991; Yura and Ogura, 2006; Ogura and Yura, 2008), la pérdida de agua (Hesp, 1991) y el exceso de radiación, especialmente cuando adquiere una coloración clara (Hesp, 1991).

Pubescencia: La adquisición de vellosidades es otra adaptación que desarrollan frecuentemente este tipo de plantas en sus superficies, principalmente en las hojas. Contribuye a la disminución de la pérdida de agua, debido a la ruptura de la continuidad entre la superficie de la hoja y la atmósfera, creando así unas condiciones intermedias más favorables para la planta (Demmig-Adams *et al.*, 1992; Daniela *et al.*, 2009; Yates *et al.*, 2010). También protege del exceso de salinidad (García-Mora *et al.*, 1999; Daniela *et al.*, 2009), de la alta radiación y temperatura (Ackerly *et al.*, 2000; Sims *et al.*, 2002; Yates *et al.*, 2010), especialmente cuando adquiere una coloración clara, y de la abrasión de la cutícula al amortiguar los impactos de las partículas de arena (García-Mora *et al.*, 1999).

Coloración de las superficies: La adquisición de una coloración clara en las superficies de las plantas es un rasgo frecuente que suele ocurrir tanto en las cutículas como en la pubescencia. Este tipo de coloración actúa incrementando el índice de reflexión de las superficies (albedo), reflejando gran parte de la radiación incidente, contribuyendo así a evitar el calentamiento causado por el exceso de radiación (Ehleringer *et al.*, 1976; Ehleringer *et al.*, 1978; Lev-Yadun, 2006; Daniela *et al.*, 2009)

Regulación estomática: Las plantas necesitan establecer un compromiso entre la adquisición de CO₂ atmosférico necesario para la fotosíntesis y la

pérdida de agua por transpiración. Esto se vuelve especialmente importante en especies de duna costera, para superar condiciones de estrés hídrico (Gratani *et al.*, 2009). Para ello, un mecanismo eficaz y que aporta una respuesta rápida, es el control de la conductividad estomática, que permite, mediante la apertura o cierre de los estomas, un mejor control del intercambio gaseoso entre la planta y la atmósfera, regulando también la temperatura foliar y la fotosíntesis (Hetherington *et al.*, 2003). Otro mecanismo eficaz, que también contribuye a esta regulación, es la densidad estomática (número de estomas por unidad de superficie), observándose en plantas bajo intensas condiciones de estrés (radiación y temperatura), una mayor densidad de estomas de pequeño tamaño (Galmes *et al.*, 2007; Fu *et al.*, 2010) que permiten un control más preciso del intercambio gaseoso entre la planta y la atmósfera.

Metabolismos alternativos tipo C₄ y CAM: Como alternativa al metabolismo C₃, algunas especies de plantas de los ecosistemas dunares costeros, en su compromiso por minimizar la pérdida de agua y tratando de mantener los niveles de carbono en el mesófilo, pueden desarrollar facultativamente alguno de los otros tipos de metabolismo como el C₄ y/o el CAM (Elhaak *et al.*, 1997; Daniela *et al.*, 2009). Las variaciones que conllevan estos metabolismos con respecto al C₃ se producen a nivel anatómico, fisiológico y bioquímico, haciendo que los procesos de captación y procesado del CO₂ se encuentren separados en el espacio y/o en el tiempo y empleando diferentes enzimas. Estos cambios les permiten a las plantas mantener más eficientemente los procesos de fijación de carbono, especialmente en ambientes de este tipo, bajo condiciones de estrés hídrico y/o salino (Elhaak *et al.*, 1997).

Asociaciones micorríticas: El establecimiento de relaciones micorríticas resulta ventajoso para las plantas, principalmente ante condiciones de escasez de agua y nutrientes esenciales. Las estructuras del hongo (micelio) actúan como una proyección de la superficie radicular de la planta (Maun, 2009; Evelin *et al.*, 2009; Garg *et al.*, 2010), incrementando la superficie de

captación. Otra de sus ventajas es que tienen la capacidad de movilizar algunos compuestos que normalmente se encuentran en formas insolubles, como ocurre con el fósforo en las condiciones particulares de estos suelos (Jakobsen, 1994; Kooijman *et al.*, 2009; Camprubi *et al.*, 2011b). La micorrización también contribuye a reducir los efectos del exceso de salinidad para la planta (Yamato *et al.*, 2008; Evelin *et al.*, 2009; Garg and Chandel, 2010). Estas asociaciones suelen ser específicas para cada especie (Cakan and Karatas, 2006; Camprubi *et al.*, 2011b) y muy dependientes de las condiciones ambientales del medio (Zhang *et al.*, 2011). En las dunas costeras predominan las micorizas arbusculares, especialmente en el frente dunar (Rodríguez-Echeverría *et al.*, 2006; Camprubi *et al.*, 2010; Camprubi *et al.*, 2011b), pero en las zonas más interiores y estabilizadas van adquiriendo cada vez mayor relevancia las asociaciones ectomicorríticas (Cakan and Karatas, 2006).

Enrollamiento foliar y cambios de orientación: Estos cambios en la disposición de la lámina foliar contribuyen a minimizar la interceptación de radiación, así como a modificar la superficie de capa límite. Actúan reduciendo los daños producidos por el exceso de radiación, consiguiendo un menor calentamiento de la superficie de la hoja y una menor pérdida de agua por transpiración (Hesp, 1991; Maun, 1994; Daniela *et al.*, 2009; Maricle *et al.*, 2009).

Suculencia: El engrosamiento foliar y la acumulación de líquido son características frecuentes en especies de ambientes dunares. Estas características le confieren a las plantas una mayor resistencia frente a distintos tipos de estrés, como el hídrico, el salino y la abrasión de sus superficies (Boyce, 1951; García-Mora *et al.*, 1999; Davy *et al.*, 2006).

Exclusión activa de sales en las raíces: En los ecosistemas dunares costeros, las plantas de las especies que adquieren este rasgo, aunque implica un mayor gasto energético tienen la ventaja de que pueden limitar selectivamente la entrada de iones a través de las raíces, que de otra forma

podrían resultar tóxicos, al alterar al equilibrio osmótico de la planta (Rozema *et al.*, 1985; Suárez, 2011).

Para contrarrestar los efectos perjudiciales que causa el exceso de salinidad, algunas especies han conseguido desarrollar ciertas adaptaciones a nivel molecular, como la síntesis de proteínas de estrés y/o osmolitos (Khedr *et al.*, 2003; Parvaiz *et al.*, 2008). Estas moléculas le permiten a la planta compensar los desequilibrios osmóticos causados por el exceso de salinidad. Otra posibilidad es la acumulación y aislamiento del exceso de sal en el interior de vacuolas, permitiendo así mantener la homeostasis en el citoplasma (Daniela *et al.*, 2009; Kosova *et al.*, 2011).

Acumulación de sal en vesículas y tricomas: Una adaptación típica de ambientes con elevada salinidad es acumular la sal en la parte exterior de la planta, bien aislándola dentro de estructuras como vesículas o tricomas (Pierce *et al.*, 2005; Voronkova *et al.*, 2008), o mediante la excreción glandular directamente sobre las superficies externas de la planta (Sánchez-Blanco *et al.*, 2003; Daniela *et al.*, 2009). La acumulación de partículas blanquecinas de sal en la superficie puede contribuir también a proteger a la planta de otros tipos de factores de estrés, como las altas temperaturas o la intensa radiación, debido al incremento de la reflexión en esas superficies (Hesp, 1991).

Variaciones en la fenología: Una gran cantidad de especies que habitan en ambientes estresantes, entre las que se encuentran muchas de las especies de ecosistemas dunares costeros, consiguen adaptar sus momentos de germinación, floración y fructificación a los periodos en que las condiciones les son más favorables, tratando a su vez de evitar los más desfavorables (Herrera, 1986; Maun, 1994), intentando maximizar el éxito de su establecimiento que probablemente sea la fase más crítica de su ciclo vital.

Adaptaciones en las semillas: Las adaptaciones a nivel de las semillas tienen gran importancia en los ecosistemas dunares costeros, donde el proceso de germinación es uno de los procesos más críticos para el desarrollo de la

planta y el éxito de la especie (Maun, 1994; Balestri *et al.*, 2004; Uysal *et al.*, 2006). Uno de los mayores limitantes se debe a las propias características del sustrato arenoso, móvil y con baja capacidad de retención hídrica. Para que las semillas logren superar el proceso de germinación, las plantas progenitoras deben acumular en ellas los recursos suficientes para soportar diferentes grados de enterramiento (Maun, 1994; Maun, 2009) y para poder activar la germinación y crecer hasta alcanzar las fuentes de recursos lo más rápidamente posible, en cuanto se produzcan las condiciones adecuadas (Maun, 1994; Balestri and Cinelli, 2004).

Integración clonal: El crecimiento clonal alcanza gran relevancia en este tipo de ambientes. Lograr superar el proceso de germinación y establecimiento es uno de los mayores desafíos en el desarrollo de la planta, debido principalmente al estrés hídrico y a la salinidad (Maun, 1994; Balestri and Cinelli, 2004). La integración clonal minimiza sus efectos y facilita la disponibilidad de recursos en los nuevos módulos en mayor medida que la capacidad que puede tener una semilla para sostener a una plántula (Evans *et al.*, 1992; Andersone *et al.*, 2011). Además, ahorra el coste energético necesario para la reproducción sexual y evita la dependencia de interacciones con los polinizadores. También es ventajoso en otros procesos como el enterramiento o la competencia, al permitir movilizar recursos y energía entre distintos módulos del mismo individuo (Maun, 2009; Roiloa *et al.*, 2010).

Forma de vida perenne: El desarrollo de una forma de vida perenne tiene para la especie un efecto similar a la integración clonal, ahorrándole a la planta el proceso de germinación y establecimiento que tienen que superar los terófitos. Asegura, en mayor medida, la prevalencia de individuos de una especie, aun a pesar de la existencia de condiciones que impidan la germinación y el establecimiento de nuevas plántulas. El mantenimiento de una estrategia perenne implica un mayor coste energético en momentos desfavorables (ej. en invierno). Sin embargo permite una respuesta más rápida y un mejor aprovechamiento de los recursos en momentos más favorables,

especialmente en ambientes altamente cambiantes e impredecibles, donde la disponibilidad de recursos es muy variable (Chesson *et al.*, 2004).

Alta plasticidad de los rasgos: La alta plasticidad es una cualidad importante para plantas de ambientes pobres y cambiantes (Maun, 2009), al permitir que un mismo individuo tenga la capacidad de vivir en un rango más amplio de condiciones (Dech and Maun, 2006; Greaver and Sternberg, 2007; Maun, 2009). La plasticidad es un rasgo importante por ejemplo ante procesos de enterramiento, ante los que las plantas reaccionan modificando su asignación de recursos hacia la elongación de tallos y formación de nuevas estructuras fotosintéticas (Gilbert and Ripley, 2008). Otro ejemplo es la capacidad para el aprovechamiento de diferentes fuentes de agua, de diversos orígenes, que contienen distintas concentraciones salinas (Greaver and Sternberg, 2007).

Como hemos visto, la gran variedad de adaptaciones que han adquirido las especies de ecosistemas dunares costeros abarcan la totalidad del rango de escalas, desde el nivel molecular y bioquímico (Hesp, 1991; Khedr *et al.*, 2003; Maseda *et al.*, 2006), hasta el nivel de comunidad y ecosistema (Rozema *et al.*, 1985; Armas and Pugnaire, 2009; Maun, 2009; Camprubi *et al.*, 2010). Las plantas adquieren estas adaptaciones para evitar o minimizar los efectos adversos de los factores de estrés y las perturbaciones, pudiendo algunas de ellas proteger ante un amplio rango de factores de estrés y perturbaciones. Como ya hemos visto, un ejemplo sería el caso de la pubescencia, que aporta protección frente a la pérdida de agua (Hesp, 1991; García-Mora *et al.*, 1999; Daniela *et al.*, 2009), el exceso de salinidad (García-Mora *et al.*, 1999; Daniela *et al.*, 2009), radiación y temperatura (Ackerly *et al.*, 2000; Sims and Gamon, 2002; Yates *et al.*, 2010), la abrasión por partículas de arena (García-Mora *et al.*, 1999), etc. Sin embargo otras adaptaciones, como por ejemplo el estado de epoxidación (EPS) del ciclo de las xantófilas, son mucho más específicas, protegiendo únicamente ante un estrés concreto, como es un exceso de radiación (Demmig-Adams, 1990;

Johnson *et al.*, 1993; Demmig-Adams *et al.*, 1996).

La acción de los factores de estrés puede empezar a ejercerse paulatinamente, o de forma repentina, como ocurre por ejemplo en un proceso de inundación por parte del agua marina o del movimiento repentino de sustrato durante procesos tormentosos (Bauer *et al.*, 2009). También es importante la duración durante la que actúa el estrés (Suárez, 2011). Ante la aparición de un factor de estrés o una perturbación, las plantas ponen en marcha una secuencia de respuestas que necesitan diferentes tiempos para su consecución (Maseda and Fernández, 2006). Por ejemplo, ante un repentino proceso de estrés hídrico la primera línea de protección que actúa son las respuestas a más corto plazo, como el cierre estomático, el plegamiento de hojas, etc. (Chaves *et al.*, 2003; Maseda and Fernández, 2006), que al tener una velocidad de respuesta más rápida aportan protección ya desde los primeros instantes. Luego, si el factor desencadenante del estrés perdura, se van incorporando paulatinamente la acción de respuestas a más largo plazo (Suárez, 2011) como son: el crecimiento de raíces, variaciones en el área foliar, un aumento de la tolerancia de los tejidos a la desecación parcial, etc. (Chaves *et al.*, 2003; Maseda and Fernández, 2006).

ZONACIÓN:

La particular distribución espacial de los factores ambientales, así como los distintos rasgos adquiridos por las diferentes especies, hacen que la vegetación muestre una distribución característica. Es esta interacción entre los factores de estrés y perturbaciones, y la tolerancia a éstos, la responsable de la característica zonación en forma de diferentes cinturones de vegetación, paralelos a la línea de costa, típica de este tipo de ambientes (Rozema *et al.*, 1985; Maun and Perumal, 1999; Dech and Maun, 2005). De entre los factores ambientales responsables de esta zonación, destacan especialmente la salinidad y la movilidad del sustrato (Fig. 2; Ishikawa *et al.*, 1995; Wilson and Sykes, 1999; Maun and Perumal, 1999; Dech and Maun, 2005; Griffiths,

2006; Gilbert *et al.*, 2008). En las zonas más próximas al mar, donde la intensidad de los factores ambientales es mayor, aparecen los primeros establecimientos vegetales. Son zonas que se encuentran en una fase incipiente del proceso de formación de dunas, caracterizadas por soportar fuertes y frecuentes procesos de perturbación que periódicamente alteran esta área (Hesp, 1991; Costa *et al.*, 1996), reiniciando continuamente el proceso. Las especies que logran ocupar esas posiciones son las que, generalmente, muestran la mayor tolerancia a las alteraciones producidas por los factores abióticos. Al establecerse son capaces de estabilizar acúmulos de sedimento, dando lugar a las primeras dunas incipientes a las que muchos autores denominan “dunas embrionarias” (Imbert *et al.*, 2001; Gagne and Houle, 2001; Houle, 2002; Honrado *et al.*, 2010). Justo a continuación, a medida que avanzamos hacia el interior, aparece el primer frente dunar, que consiste en una acumulación de sedimento arenoso que forma una barra más o menos continua distribuida en paralelo a la línea de costa. En esta posición ya nos encontramos un menor grado de perturbación y por tanto una mayor estabilidad del sustrato, sin embargo, aún sigue experimentando recurrentes episodios de perturbación por la acción del viento y del mar, cuya influencia se va atenuando a medida que avanzamos hacia el interior. A su vez, va siendo colonizada por un cada vez mayor número de especies debido a las menores exigencias ambientales. Algunos autores denominan a las áreas no consolidadas de la duna, en las que estarían incluidas las dunas embrionarias y el frente dunar contiguo, como “duna primaria” (Franks *et al.*, 2003; Frederiksen *et al.*, 2006) debido a su dinámica e inestabilidad características. A las posiciones más interiores, cada vez con mayor estabilidad del sustrato y cobertura vegetal, se las denomina “duna secundaria” (García-Mora *et al.*, 1999; Kim *et al.*, 2009). A medida que avanzamos hacia el interior, la vegetación va pasando de estar compuesta por especies más tolerantes a las restricciones de origen abiótico, a otras mejor adaptadas a las de origen biótico, especialmente la competencia (Maun, 1994; Ishikawa *et al.*, 1995;

Martínez and García-Franco, 2008). A medida que nos alejamos de la costa, además de los cambios de las características de las especies, se observa un incremento en la diversidad y cobertura vegetal, cambiando la estructura de la vegetación, que pasa de ser predominantemente herbácea a arbustiva, culminando finalmente en la arbórea (bosque) en las posiciones más interiores y estables (Griffiths, 2006).

La presencia o ausencia de los distintos tipos de especies a lo largo del gradiente, depende de la actuación de diferentes mecanismos según normas de ensamblaje (Diamond, 1975) recogidos en la teoría de “ensamblaje de comunidades” (Diamond, 1975; Weiher *et al.*, 1999; Cornwell *et al.*, 2009). Según esta teoría, la formación de las comunidades es un proceso dinámico en el que las especies son seleccionadas atendiendo a las características de sus rasgos funcionales. Esto ocurre mediante la acción de dos grandes fuerzas que actúan simultáneamente pero con tendencias opuestas, generando procesos convergentes y procesos divergentes (Grime, 2006; Mouillot *et al.*, 2007; Pillar *et al.*, 2009). Normalmente los procesos convergentes son explicados mediante la teoría de los “filtrado ambiental” (Keddy, 1992; Díaz *et al.*, 1998; Weiher and Keddy, 1999; Mouillot *et al.*, 2007; Silva *et al.*, 2009; Mayfield *et al.*, 2009), que se fundamenta en que las presiones abióticas actúan limitando el rango de rasgos funcionales válidos que pueden tener las especies. Esto provoca una convergencia de los rasgos y estrategias de las especies que logran coexistir en un mismo lugar, permitiendo así el establecimiento de unas determinadas especies e impidiendo el de otras (Fig. 3). Algunos ejemplos serían lo que ocurre en las especies leñosas perennes de climas mediterráneos, donde predominan las especies esclerófilas de hojas pequeñas y gruesas (Ackerly, 2004; Gratani *et al.*, 2006), las plantas de zonas alpinas con una gran tolerancia a la congelación (Cornwell *et al.*, 2006), o las plantas de zonas desérticas con adaptaciones a las altas temperaturas y la sequía prolongada, bien sea adquiriendo características tipo cactus, mostrando succulencia, hojas espinosas, metabolismo de tipo CAM, etc., o bien como terófitas efímeras de

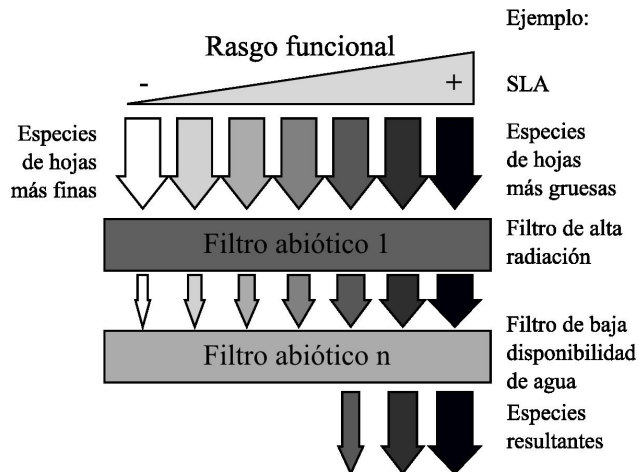


Fig. 3. Esquema del proceso de filtrado ambiental. Se representa el rango de variaciones para un rasgo funcional y como éste se ve reducido al estar sometido a diversos filtros ambientales. Finalmente, el número de especies resultantes es más restringido que las iniciales, tendiendo a converger hacia unas características determinadas del rasgo.

rápida germinación y ciclo reproductivo, condicionado por los escasos episodios de precipitación (Mouillot *et al.*, 2007).

Por otro lado, los procesos divergentes generalmente son explicados principalmente mediante las teorías de “segregación de nichos” y del “límite de similitud” (MacArthur *et al.*, 1967; Stubbs *et al.*, 2004; Mouillot *et al.*, 2007). Ambas se fundamentan en el establecimiento de procesos competitivos y proponen que la coexistencia es posible siempre y cuando las especies no tengan solapadas sus necesidades en cuanto al uso de recursos y muestren rasgos diferenciados (Fig. 4). En caso contrario estas teorías vaticinan fenómenos de exclusión debido a procesos competitivos, impidiendo que ambas especies puedan coexistir y/o disminuyendo sus abundancias. Sin embargo, aun bajo condiciones de competencia, Chesson *et al.* (2004) propusieron, para el caso particular de condiciones cambiantes, una “teoría general de competencia y coexistencia en ambientes variables”, tomando

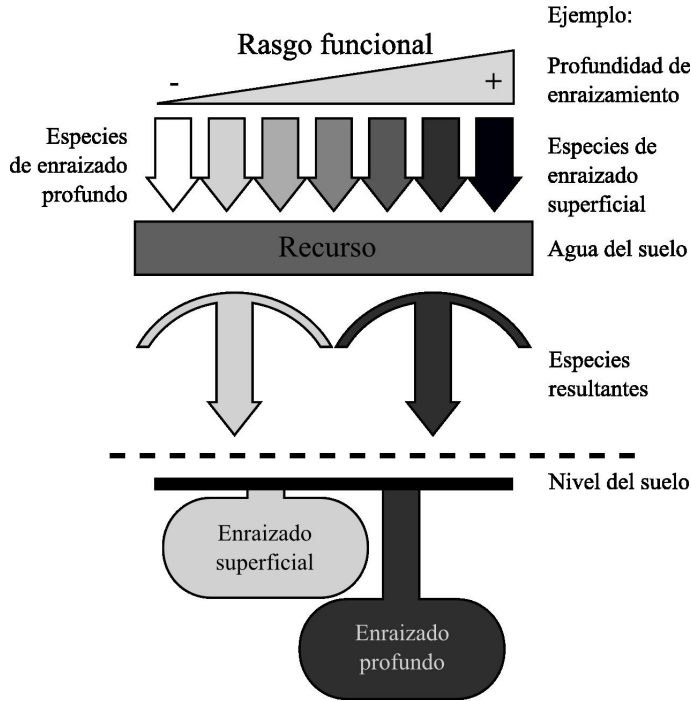


Fig. 4. Esquema del proceso de límite de similitud. Se representa el rango de variaciones para un rasgo funcional y como la competencia por un recurso hace que únicamente puedan coexistir las especies con las características más contrastadas para ese rasgo. En la parte inferior se muestra el ejemplo concreto de la diferente profundidad de enraizamiento causada por la competencia por el agua.

como ejemplo a dos especies de plantas del desierto que coexisten en competencia bajo pulsos variables de precipitación. En esta teoría destacan principalmente dos procesos, que son responsables de la coexistencia de diferentes especies en las comunidades, basados en la existencia de diferencias en los patrones de crecimiento poblacional y consumo de recursos entre especies a lo largo del tiempo. Se denominaron “efecto almacenamiento” y “no linealidad relativa de la competencia”. El “efecto almacenamiento” consiste en que una especie puede crecer y captar recursos en los periodos más

favorables para la especie, de menor competencia, y permanecer latente en los periodos menos favorables de mayor competencia (Chesson, 2000; Chesson *et al.*, 2004). Las distintas especies muestran momentos óptimos diferentes de captación de recursos a lo largo de su fenología o ciclo vital. La “no linealidad relativa de la competencia” se basa principalmente en las distintas capacidades que muestran las diferentes especies para explotar los distintos patrones de disponibilidad de recursos que se pueden producir, ya sea una sucesión de pequeños pulsos de precipitación, o bien pulsos más prolongados de precipitación más abundante, penetrando más profundamente en el suelo y con un mayor tiempo de permanencia. Algunas especies invierten recursos en mantener estructuras, como raíces u hojas, que le permiten responder más rápidamente o durante un mayor periodo de tiempo ante la aparición de un recurso, así como explotar pulsos más cortos y/o de menor intensidad. Otras especies responden solamente ante pulsos más prolongados y de mayor intensidad, necesitando mayores tiempos de respuesta para la formación de estructuras pero mostrando una mayor capacidad para explotar los recursos más intensamente.

Alternativamente, Grime (2006) plantea otros dos tipos de filtros para explicar los procesos de convergencia y divergencia de rasgos y especies relacionados con factores abióticos en lugar de procesos competitivos. Por un lado describe los “filtros de productividad” que contribuyen a explicar la convergencia entre rasgos y/o especies. Se definen como aquellos procesos que afectan a la producción de biomasa, almacenamiento de carbono, ciclo de los nutrientes, protección contra herbívoros, etc. Por otro lado, los “filtros de perturbación” contribuyen a explicar los procesos de divergencia. Existen multitud de posibles episodios de perturbación que son ampliamente variables en el espacio y en el tiempo, permitiendo así promover y mantener la diversidad de rasgos funcionales y especies en las comunidades vegetales. A una escala local, Grime considera a la perturbación como el principal impulsor de la diferenciación de rasgos y la coexistencia de las especies (Grime, 2006).

La presencia o ausencia de unas especies u otras a lo largo del gradiente dunar depende del equilibrio que se produzca entre estas dos grandes fuerzas, prevaleciendo en cada lugar únicamente las especies capaces de superar todo este tipo de filtros y restricciones que generan.

ESPECIES DE ESTUDIO:

Para la realización de este estudio se hizo una selección entre las especies más abundantes y características de los ambientes dunares costeros. De entre éstas, se eligieron un conjunto de especies herbáceas perennes que crecen en la duna embrionaria y el frente dunar, aunque algunas de ellas lograron distribuirse a lo largo de un mayor rango de distancias, colonizando posiciones más internas a lo largo del gradiente dunar. Las especies seleccionadas fueron:

Calystegia soldanella (L.) R. Br. (Convolvulaceae) (Fig. 5). Es una planta



Fig. 5. Ejemplar de *Calystegia soldanella* (L.) R. Br. (Convolvulaceae).

herbácea vivaz, de tallos procumbentes de 10-100 cm de longitud. Glabra, con hojas enteras arriñonadas y algo carnosas. Tiene peciolo largo de unos 10 cm y orejuelas en la inserción. Vive en dunas y arenales costeros entre 0 y 10 m sobre el nivel del mar, en zonas de pH neutro y nitrificadas. Se distribuye desde Dinamarca y Escocia hasta Turquía, ocupando también las islas del Mediterráneo. Se encuentra además en América (Sur y norte), África, Asia y



Fig. 6. Ejemplar de *Eryngium maritimum* L. (Umbelliferae)

Australia. Presenta tanto reproducción sexual como asexual. La asexual es mediante rizomas, produciendo módulos. La reproducción sexual se caracteriza por ser hermafrodita. Tiene flores solitarias acampanadas, de unos 6 cm de longitud, con cinco pétalos soldados rosas con estrías blancas. La floración comienza en mayo y dura todo el verano. El tipo de polinización es entomófila. Es una especie hemicriptófita, con un metabolismo de tipo C₃, pudiendo desarrollar facultativamente el tipo C₄.

Eryngium maritimum L. (Umbelliferae) (Fig. 6). Planta vivaz de 15-60 cm de altura, con hojas glabras espinosas de cutícula dura ambas con función defensiva, de color verdoso a gris claro. Tiene enraizamiento profundo (mayor de 1 m). Las flores se agrupan en glomérulos compactos de color azulado, mientras que los frutos son escamosos. Ambos presentan espinas. Se desarrollan en dunas primarias y secundarias. En Europa se distribuyen desde



Fig. 7. Ejemplar de *Matthiola sinuata* (L.) R. Br. (Brassicaceae)

Noruega hasta Turquía, ocupando las islas mediterráneas. También se puede encontrar en África y Asia. Por su reproducción sexual, es hermafrodita. La floración se produce entre julio y octubre, y la polinización es entomófila. Los frutos son de unos 3 mg y presentan hidrocoria y anemocoria y germinan en primavera y verano. Es una especie hemicriptófito, con un metabolismo de tipo C₃.

Matthiola sinuata (L.) R. Br. (Brassicaceae) (Fig. 7). Planta perenne (o

bienal) de 20 -60 cm. Posee hojas oblanceoladas u oblongo-elípticas, pinnatífidas o enteras. Las inferiores son algo mayores que las superiores. Presentan una densa pubescencia, formada por pilosidades ramificadas y pelos glandulares, que le aporta una coloración blanquecina. Su distribución es principalmente europea (oeste y sur) aunque también se puede encontrar en algunos puntos del norte de África. Sus flores son de color morado claro y se



Fig. 8. Ejemplar de *Euphorbia paralias* L. (Euphorbiaceae)

agrupan en racimos. Florece entre marzo y septiembre y su polinización es entomófila. Es una especie hemicriptófita con metabolismo tipo C_3 .

Euphorbia paralias L. (Euphorbiaceae) (Fig. 8). Planta perenne, rizomatosa, glabra de coloración más o menos glauca. Presenta múltiples tallos que pueden llegar a unos 80 cm de altura, con base leñosa y cicatrices de inserciones foliares. Los tallos presentan ramificaciones que pueden ser tanto fértiles como estériles. Las hojas se sitúan hacia el ápice, son enteras, simples,

apuntadas y sésiles. Son lustrosas en el haz y glaucas en el envés. Las raíces alcanzan cierta profundidad (más de 1 m). Se desarrolla en playas y sistemas dunares. En Europa se distribuye desde los Países Bajos hasta Turquía, encontrándose también en las islas del Mediterráneo y África. No produce módulos. Su reproducción es de tipo sexual, formándose las flores en las ramificaciones fértiles. Los frutos son de unos 5 mm y las semillas ovoideas



Fig. 9. Ejemplar de *Pancratium maritimum* L. (Amaryllidaceae).

de unos 4.7 mg. Florece entre julio y octubre y la polinización es entomófila. La germinación se produce en primavera. Su forma vital es caméfito y el metabolismo es de tipo C₃, pudiendo desarrollar facultativamente el C₄ y CAM.

Pancratium maritimum L. (Amaryllidaceae) (Fig. 9). Planta perenne bulbosa, con un bulbo blanquecino bajo el cual se extienden las raíces que pueden alcanzar los 80 cm de profundidad. De la base salen 4-6 hojas glabras,

carnosas y glaucas de hasta 60 cm de altura y 1.5 cm de ancho. Las flores son pediceladas blancas y pueden alcanzar los 15 cm. Habita playas y dunas tanto primarias como secundarias. En Europa se distribuye desde Bretaña y el sur de las Islas Británicas hasta Turquía por toda la zona mediterránea, habitando además en el norte de África. Su reproducción puede ser asexual, por bulbos, o sexual. Las flores se encuentran agrupadas, de 4 a 12, en umbelas en el



Fig. 10. Ejemplar de *Honckenya peploides* (L.) Ehrh. (Caryophyllaceae).

extremo de escapos de 45 cm. Los frutos son cápsulas subglobosas que contienen semillas aplastadas y brillantes. La floración es tardía. Su forma vital es geófito y tiene un metabolismo de tipo C₃.

Honckenya peploides (L.) Ehrh. (Caryophyllaceae) (Fig. 10). Planta herbácea perenne hemicriptófito (rizomatosa). Tiene estructuras aéreas suculentas de 10 a 25 cm de altura, con hojas gruesas de forma ovada a elíptica dispuestas perpendiculares a lo largo del tallo (90°), de color verde a

amarillo-verdoso. Es una especie dioica, más concretamente subdioica - un punto intermedio entre el hermafroditismo y la dioecia -, pues algunas flores macho tienen la capacidad de producir frutos y semillas (viables). Tienen pequeñas flores blancas actinomorfas que crecen en solitario en el extremo de los tallos y producen néctar. Desarrollan dos tipos distintos de flores, las que se corresponden con las hembras (denominadas “pistiladas”) muestran pequeños pétalos, largos estilos, anteras no funcionales - por lo que nunca producen polen - y muestran constancia en su expresión. Las correspondientes a los machos (denominadas “estaminadas”) desarrollan mayores pétalos, estilos más cortos y anteras productoras de polen. Florecen entre marzo y junio y fructifican desde mayo hasta finales de agosto. También presenta reproducción asexual a partir de rizomas. Crece en el límite entre la playa y la duna primaria (duna embrionaria) formando manchas, frecuentemente unisexuales no clonales. En la península se distribuye desde Lisboa hacia el norte, ocupando toda el área del Cantábrico, continuando por el resto de Europa hasta Noruega, alcanzando a nivel mundial una distribución circumpolar y encontrándose también introducida en algunos lugares de América del Sur. Su metabolismo es de tipo C₃.

SITUACIÓN GEOGRÁFICA:

Los tres sistemas dunares costeros estudiados están distribuidos a lo largo de la costa de la comunidad autónoma de Galicia (NO de España; Fig. 11). En este territorio confluyen dos de las grandes regiones bioclimáticas, la atlántica y la mediterránea, apareciendo características intermedias entre ambas. Las localidades fueron seleccionadas atendiendo a varios criterios como son: la presencia de las especies, su distribución espacial en el ambiente, ya fuese coexistiendo en un mismo espacio o distribuyéndose a lo largo de un gradiente, y su estado de conservación, tratando de seleccionar los menos alterados.

El sistema dunar de Valdoviño (Fig. 12 superior; 43°36'44"N, 8°10'58"O)

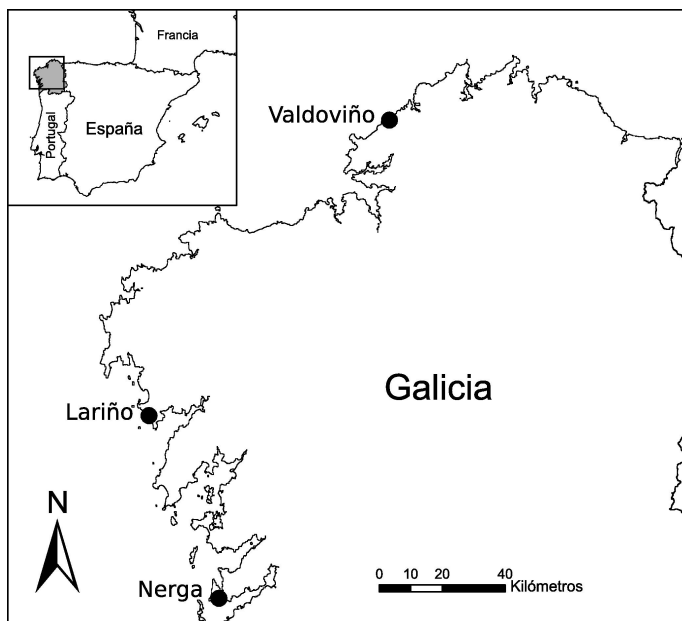


Fig. 11. Localización geográfica de los diferentes sistemas dunares estudiados en este trabajo.

muestra una orientación norte, con una pendiente del 5%. En el extremo oeste, se encuentra su zona mejor conservada, y en ella encontramos poblaciones de las especies *P. maritimum* y *E. maritimum*, distribuidas a lo largo de un gradiente ambiental costa-interior de 600 m de gran interés para el estudio.

El sistema dunar de Nerga (Fig. 12 inferior; 42°15'50"N, 8°51'10"O) es el que se encuentra situado más al sur, en la cara norte de la entrada de la ría de Vigo. Muestra una orientación sur, con una pendiente del 3%. En este sistema, podemos encontrar una población de *P. maritimum* distribuida a lo largo de un gradiente costa-interior de 800 m.

En una posición central entre las anteriores, se encuentra el sistema dunar de Lariño (Fig. 13; 42°46'00"N, 9°06'58"O). Muestra una orientación norte,

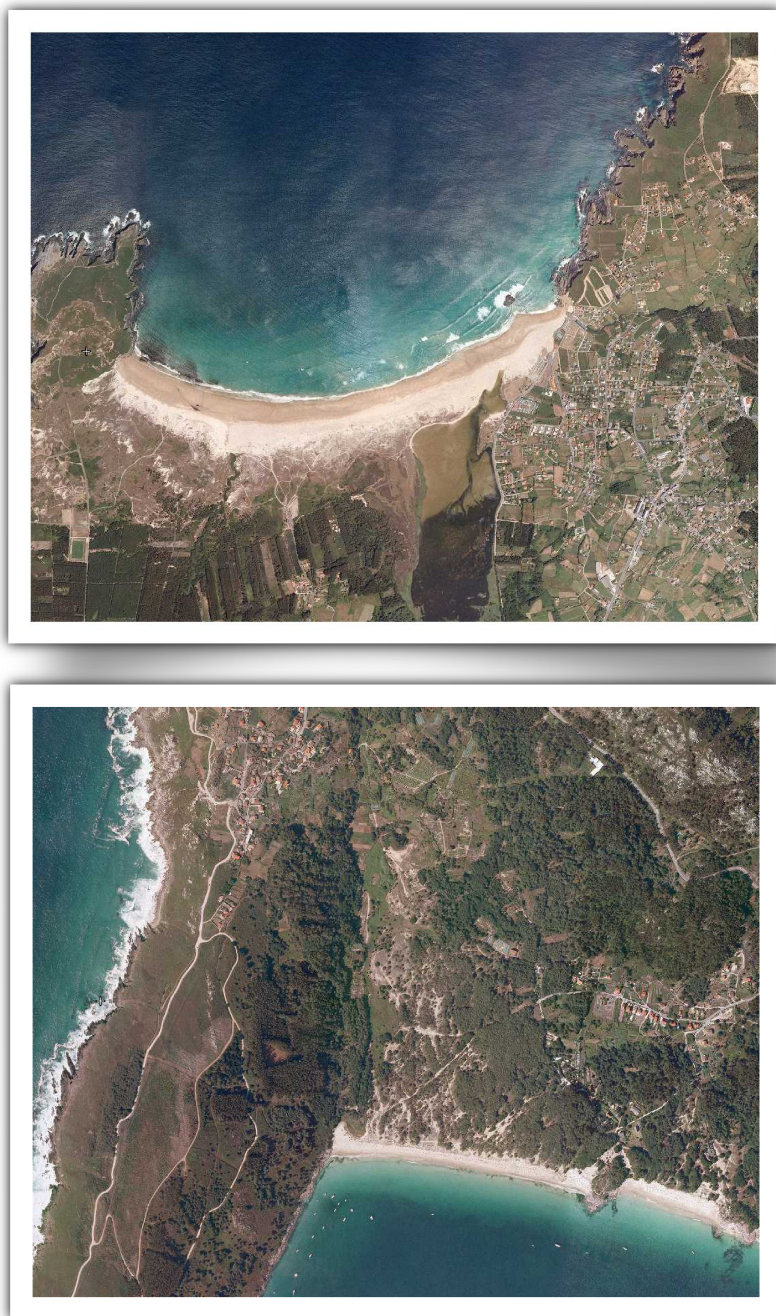


Fig. 12. Vista aérea de los sistemas dunares de Valdoviño (superior) y Nerga (inferior).

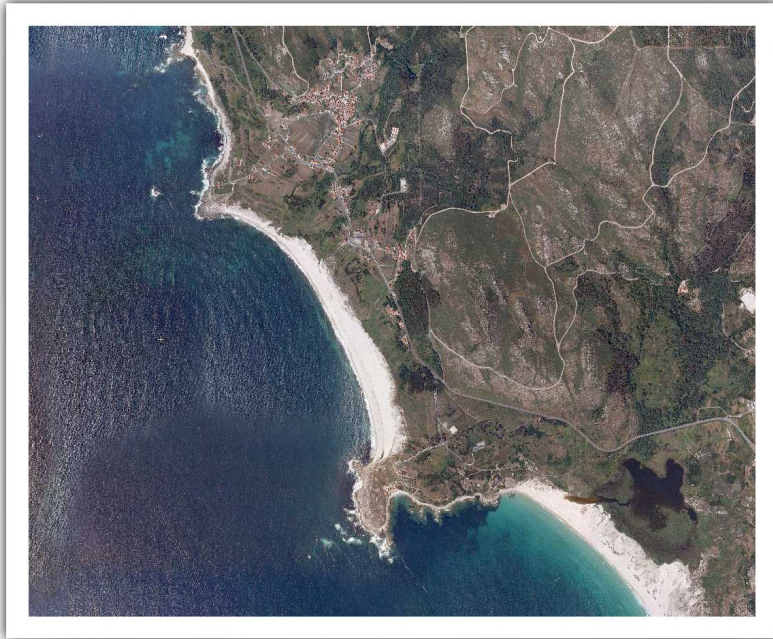


Fig. 13. Vista aérea del sistema dunar de Lariño.

con una mínima pendiente. En la duna primaria de su parte central, coexisten, mezcladas homogéneamente, cinco especies de plantas interesantes para el estudio: *M. sinuata*, *E. paralias*, *E. maritimum*, *C. soldanella* y *P. maritimum*. A lo largo de toda la parte superior de la playa, justo debajo del frente dunar (duna embrionaria), se encuentran manchas de *H. peploides*, la mayor parte de ellas unisexuales, con manchas de ambos sexos alternadas entre sí.

OBJETIVOS

Una característica bien conocida de los ambientes dunares costeros es la gran variabilidad y divergencia de los rasgos relacionados con la morfología foliar y las formas de crecimiento de las especies de plantas que allí habitan.

Sin embargo, resulta mucho menos conocido qué ocurre a nivel ecofisiológico en rasgos relacionados con el rendimiento, especialmente en condiciones naturales. Para avanzar en el conocimiento de estos aspectos, este trabajo se propuso como objetivo general el comprobar si esa variabilidad, a nivel de morfología foliar y formas de crecimiento, se ve reflejada también a nivel ecofisiológico en rasgos de rendimiento, o si por contra estos rasgos resultan ser menos variables y más conservados. La función fotosintética es crucial para las plantas, y en general para el funcionamiento de los ecosistemas tal y como los conocemos. Su variabilidad podría verse aún más restringida debido a las fuertes constricciones causadas por los factores ambientales extremos característicos de este tipo de ecosistemas. Se ha sugerido que en ambientes donde se producen simultáneamente importantes restricciones ambientales y un elevado nivel de perturbaciones, no hay estrategias posibles para la supervivencia de las plantas. Los ecosistemas dunares costeros pueden presentar simultáneamente ambas características, por lo que resulta de especial interés conocer las estrategias que las plantas han desarrollado para subsistir en estos ambientes. Este es otro de los objetivos principales que nos propusimos responder en este estudio. La consecución de estos objetivos principales requirió que nos planteásemos una serie de objetivos más específicos, que mediante la aplicación de diferentes enfoques y perspectivas se abordaron en los diferentes capítulos de este trabajo. Para ello seleccionamos y estudiamos diferentes rasgos funcionales relacionados con el rendimiento, claves para el desarrollo de las especies de sistemas dunares costeros, atendiendo a su variabilidad inter e intraespecífica y a diferentes escalas temporales y espaciales.

Pretendemos, de esta forma, conocer el comportamiento de diferentes rasgos funcionales que integran información de las condiciones a las que se vieron sometidas las plantas entre cierto momento del pasado y la actualidad, integrando información del estado de la planta durante diferentes rangos temporales: a más largo plazo (escala interanual, estacional a mensual),

intermedio (desde varias semanas a horas) hasta a un plazo más corto (desde unos pocos minutos a cambios prácticamente instantáneos).

Desde el punto de vista de las especies, pretendemos conocer tanto su variabilidad intraespecífica, buscando posibles diferencias incluso entre los diferentes sexos, como la variabilidad interespecífica, estudiando el comportamiento entre las diferentes especies seleccionadas.

Desde el punto de vista de la variabilidad temporal, pretendemos conocer la variación en el comportamiento de las especies a diferentes escalas temporales, desde la variabilidad interanual, pasando por la estacional, mensual, e incluso variaciones en el rango de unos pocos minutos o hasta prácticamente instantáneas.

Desde el punto de vista de la variabilidad espacial, pretendemos comprender desde el comportamiento y variabilidad en plantas que coexisten en áreas reducidas y homogéneas, de unos pocos metros cuadrados, hasta la variabilidad entre distintas posiciones que ocupan las especies que logran distribuirse a lo largo de gradientes costa-interior de varios centenares de metros.

Para ello:

En el *capítulo 1* estudiamos la variabilidad en rasgos funcionales que integran información a escalas amplias (estaciones, meses). Concretamente abordamos las variaciones de varios recursos fundamentales para la planta, como son el agua y el nitrógeno. Lo hacemos principalmente a partir del estudio de la composición de isótopos del carbono y nitrógeno, pero también de su densidad estomática, área específica foliar, etc. Este estudio lo realizamos comparando entre diferentes especies que coexisten en una área reducida, comparando la información entre dos estaciones contrastadas (estival e invernal) y tratando de identificar las diferentes estrategias que siguen las especies, así como su tendencia (convergencia-divergencia).

En el **capítulo 2** estudiamos el la variabilidad de rasgos funcionales que aportan información integrada en un rango temporal intermedio (semanas o meses). Para ello estudiamos el contenido en agua y pigmentos, relacionados con la captación y disipación de la radiación, así como la eficiencia fotoquímica, a partir de técnicas de reflectancia espectral y fluorescencia clorofílica. Este estudio lo realizamos entre diferentes especies que coexisten en una área reducida y homogénea del frente dunar, comparando el comportamiento entre estaciones contrastadas (estival e invernal) y entre diferentes meses dentro de una estación (verano). Se tratan de discernir también las estrategias desarrolladas por las diferentes especies intentando identificar posibles procesos de convergencia o divergencia entre ellos.

En el **capítulo 3** estudiamos la variabilidad intra e interespecífica de rasgos funcionales, relacionados con el rendimiento fotosintético y la adquisición de recursos como agua y nutrientes, y las principales características edáficas. Para ello se emplean principalmente técnicas basadas en la reflectancia espectral y fluorescencia clorofílica, además de la discriminación isotópica y otras basadas en la estructura foliar, que integran información del estado de la planta a medio y más largo plazo. Concretamente pretendemos conocer el comportamiento de dos especies, y sus interacciones con varios de los principales parámetros fisicoquímicos del suelo, tratando de identificar los posibles procesos convergentes y/o divergentes que puedan ocurrir tanto entre ambas como entre las diferentes posiciones a lo largo de un gradiente costa-interior en el que se distribuyen.

En el **capítulo 4** estudiamos las respuestas de las especies dunares a rasgos funcionales relacionados con la eficiencia del uso de la radiación. Para ello empleamos técnicas basadas en la fluorescencia clorofílica, que integran información a medio (semanas) y más corto plazo (minutos a prácticamente instantáneo). Con estas técnicas pretendemos conocer tanto el estado actual de las plantas en cuanto a su capacidad de procesar radiación, su capacidad de responder a cambios rápidos en la dosis de radiación, así como su facilidad

para recuperar su capacidad de procesamiento de luz tras verse sometidas a altas dosis de radiación. Además pretendemos conocer también tanto su variabilidad temporal, comparando para dos estaciones contrastadas (estival e invernal), como espacial y específica, estudiando las respuestas de diferentes especies que coexisten en una área reducida y homogénea o de una única especie que se distribuye a lo largo de un gradiente costa interior. Con todo esto pretendemos también reconocer y comparar las posibles diferencias en las estrategias que puedan existir entre diferentes especies o posiciones a lo largo de un gradiente.

En el *capítulo 5* se estudia la variabilidad intraespecífica entre los diferentes sexos de una especie, midiendo y comparando sus capacidades para captar y acumular recursos en estructuras subterráneas (raíces y rizomas), aéreas y reproductivas. Para ello empleamos variables que integran información de la situación a la que estuvo sometida la planta en un periodo medio o largo. Concretamente estudiaremos el contenido hídrico del suelo y la biomasa acumulada en los diferentes compartimentos y a diferentes profundidades, comparando tanto su variabilidad interanual como mensual, a lo largo de dos años consecutivos.

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Chapter **1**.

Together...but different: co-occurring dune plant species differ in their water and nitrogen use strategies.

ABSTRACT

1. Community assembly studies have suggested that stress factors may severely constrain the range of plant physiological responses in harsh environments. Convergence of traits is expected in coastal dunes because of environmental filtering processes imposed by severe abiotic factors. However, the wide range of morphological and phenological traits exhibited by dune species suggests that limiting similarity and/or disturbance processes generate considerable trait divergence. Thus, we hypothesized that plant survival in these environments may be achieved by different strategies.
2. Five species with different functional and morphological traits, but that were coexisting in a homogeneous area of a coastal dune in NW Spain, were selected for study. Soil characteristics and leaf functional traits

were measured in April, June and November 2008.

3. Integrated water use efficiency (assessed by carbon isotope discrimination: $\Delta^{13}\text{C}$) and nitrogen acquisition and use strategies (estimated by nitrogen isotope composition: $\delta^{15}\text{N}$) varied significantly among species and the differences changed significantly over time. Species differences in other traits, such as specific leaf area, relative water content, leaf nitrogen and carbon to nitrogen ratio, also varied over time. The species differed in stomatal density but not in the associated soil characteristics, with the exception of pH.
4. The species coexisting in the foredune system under study widely differed in several strategic functional traits related to the use of essential resources such as water, nitrogen and light, suggesting species niche segregation. Species-specific temporal effects on the use of these resources support temporal niche differentiation. Somewhat in contrast to the findings of previous studies on harsh environments, the present findings revealed a considerable level of functional diversity and complexity, suggesting that limiting similarity and/or disturbance processes may be important in structuring the dune community under study.

KEYWORDS: Functional trait divergence, isotopic composition, limiting similarity, niche differentiation, species coexistence, water use efficiency.

INTRODUCTION

Plants that grow in coastal sand dunes, especially foredune plants, are exposed to very harsh and unpredictable environmental conditions. Water and nitrogen are among the most important limiting factors for vegetation in sand dune ecosystems (Tilman, 1987; Sharifi *et al.*, 1988), but other stress factors such as high temperature and light intensity (Barbour *et al.*, 1985; Hesp, 1991;

Davy *et al.*, 1993), wind exposure (Maun, 2009), sand burial (Maun *et al.*, 1999), salt spray and soil salinity (Wilson *et al.*, 1999) are liable to limit plant productivity and to determine community structure. The very stressful conditions imposed by the concurrence of these environmental factors, operating at variable temporal and spatial scales, coupled with the high degree of disturbance and unpredictability of these environments (Robinson *et al.*, 1983; Crawford, 2008), explain why only some plant species with particular adaptations are able to survive in such hostile environments (Gray, 1985; Hesp, 1991; Maun, 1994; Maun, 1998). Such adaptations occur at multiple levels, ranging from biochemical and physiological (Khedr *et al.*, 2003; Daniela *et al.*, 2009) to phenological and ecological (Armas *et al.*, 2009).

Much research has focused on providing explanations for the maintenance of the diversity found in dune systems, and two main processes are considered: environmental filtering and limiting similarity (MacArthur and Levins, 1967; Meinzer, 2003; Fukami *et al.*, 2005; Grime, 2006; Pillar *et al.*, 2009). However, studies in other types of ecosystems have emphasized the diversifying effect of disturbance (Grubb, 1977; Grime, 2006). Many community assembly studies have suggested that in harsh environments, such as sand dune ecosystems, stress factors may severely constrain the range of plant physiological responses (Reich *et al.*, 1997; Paruelo *et al.*, 1998; Reich *et al.*, 2003; Meinzer, 2003; Bucci *et al.*, 2004; Swenson *et al.*, 2007) in a process referred to as environmental filtering (Abrams, 1983; Houle, 1996; Webb *et al.*, 2002; Cornwell *et al.*, 2006; Grime, 2006; Mouillot *et al.*, 2007). As a consequence of this process, the strategies of plants growing under the hostile conditions of such environments may converge to produce a specific set of functional traits, in a phenomenon named functional convergence (Blackman *et al.*, 2005; Jacobsen *et al.*, 2008). For example, the functions of succulent tissues (to store water) and of pubescent epidermis and a thick cuticle (to reduce transpiration and water loss) have long been considered as adaptations to coastal sand dune environments (Hesp, 1991; Maun, 2009).

Morphological and physiological similarities among plants growing in the same types of environment have been considered a paradigm of convergent evolution (Cody *et al.*, 1978). According to Ackerly (2004), convergence occurs as the result of the action of natural selection, because only this evolutionary force could generate similar phenotypes under similar conditions in independent locations and lineages. On the other hand, in environments where the main stress factors are related to density and competitive processes rather than to physical factors, limiting similarity (i.e. the maximum similarity in resource use patterns that is consistent with the coexistence of two or more competing species) has been proposed as an important process that shapes community structures (MacArthur and Levins, 1967; Abrams, 1983; Mouillot *et al.*, 2007). Consequently, species are able to coexist stably because they differ in their ecology in some particular way, for example in the time of maximal resource use (Chesson *et al.*, 2004). Because of the harsh conditions imposed by severe abiotic factors in coastal dunes, we would mainly expect species to converge to a specific configuration of traits imposed by environmental filtering, but we would also expect a considerable degree of trait divergence, driven by limiting similarity and/or disturbance processes within such plant communities. In fact, some authors have reported lower co-occurrence than expected by chance in sand dune species in New Zealand (Stubbs and Wilson, 2004) and Portugal (Maltez-Mouro *et al.*, 2010).

Although the functional fit between organisms and their environments is an essential feature of biological diversity (Ackerly, 2004), little is known about this subject in coastal dune systems, and direct comparisons of physiological differences between co-occurring dune species are scarce (Castillo *et al.*, 2002; Rubio-Casal *et al.*, 2010). This is an important issue to address in order to understand differences in the responses of these species to key environmental factors. Knowledge of these differences will improve our understanding of the effects of predicted global changes on species distribution in environments where the severity of abiotic stresses, in terms of

intensity, frequency and unpredictability, may increase as a result of global warming (IPCC, 2004; Loik *et al.*, 2004). In this study, we applied a comparative approach to investigate some key components of the strategies used by five co-occurring foredune species to cope with the challenge of surviving in such unfavourable disturbed environments. The species *Calystegia soldanella*, *Euphorbia paralias*, *Eryngium maritimum*, *Pancratium maritimum* and *Matthiola sinuata* were chosen because of their ubiquitous distribution in the coastal dune systems of NW Spain and their presence on foredunes where both resource stress and disturbance are particularly severe. These species exhibit very different life-history and leaf traits, but little is known about the ecophysiological differences between them. Considering the wide range of morphological and phenological traits displayed by these species, we hypothesized that survival in these environments can be achieved by different strategies resulting from the combination of different adaptations, which suggests that there is more than one biotic solution to the same abiotic conditions. To test this hypothesis, we explored whether the species differed in how efficiently they use two essential resources: water and nitrogen. Specifically, we focused on the integrated water use efficiency (WUE), assessed by carbon isotope discrimination ($\Delta^{13}\text{C}$) and integrated nitrogen use and acquisition strategies, as estimated from nitrogen isotope composition ($\delta^{15}\text{N}$) (Robinson *et al.*, 2000). $\Delta^{13}\text{C}$ provides information integrated over extended periods about processes such as leaf conductance, hydraulic capacity, potential WUE and photosynthetic capacity (Körner *et al.*, 1988; Farquhar *et al.*, 1989). $\delta^{15}\text{N}$ has been used less extensively in plant ecophysiology, but has been proposed as an integrative trait to assess stress tolerance in plants (Robinson *et al.*, 2000; Lopes *et al.*, 2006) and to study integrated plant N acquisition, assimilation and use (Filella *et al.*, 2003). We also analysed species variability in soil characteristics and relevant leaf traits such as carbon, nitrogen and water content, specific leaf area (SLA: leaf area / leaf mass), which is related to photosynthetic capacity, leaf longevity and stress tolerance

(Westoby, 1999), and stomatal density, which contributes to stomatal conductance, which in turn regulates the diffusion of CO₂ into the leaf during photosynthesis and the outward diffusion of water vapour (Hetherington and Woodward, 2003). Analysis of these variables enabled us to explore whether species differed in other strategic morpho-functional traits that may play a crucial role in the segregation of their ecological niches and therefore in explaining stable coexistence of species.

MATERIAL AND METHODS

Species and site

Five co-occurring perennial herbaceous species, which are common in the coastal dunes of Galicia (NW of Spain), were selected for study. These species belong to five different families and display very different functional and morphological leaf characteristics. *Eryngium maritimum* L. (Umbelliferae) is a hemicryptophyte with three-lobed folded leaves. The plant has stiff, spiny leaves, which have a leathery texture and are bluish-green in colour. *Euphorbia paralias* L. (Euphorbiaceae) is a chamaephyte with fertile and sterile branches and closely overlapping leaves. The leaves are of mean size 20 mm and are an ovate to elliptical in shape. *Pancratium maritimum* L. (Amaryllidaceae) is a cryptophyte (bulbous plant). A long neck grows from bulb and ends in linear-shaped glaucous leaves. *Matthiola sinuata* (L.) R. Br. (Brassicaceae) is a hemicryptophyte that grows as a basal rosette. The leaves and stems are covered with thick gray down. *Calystegia soldanella* (L.) R. Br. (Convolvulaceae) is a hemicryptophyte with fleshy, kidney- or heart-shaped leaves. It also has large trumpet-shaped, pink to purplish pink flowers with lighter stripes. All these species have a C₃ metabolism, although *C. soldanella* and *E. paralias* can switch to C₄ metabolism and *E. paralias* also to CAM metabolism, depending on habitat factors (Elhaak *et al.*, 1997; Daniela *et al.*,

2009). All selected species are amphistomatic, except *E. paralias*, which is epistomatic (Daniela *et al.*, 2009).

In the coastal dune at Lariño (Galicia, NW Spain, 42°46'00"N, 9°06'58"W), we delimited an apparently homogeneous area of 220 m² in the foredune. Within this area, twelve different individuals of each species were sampled in April, June and November of 2008.

Leaf Relative Water Content, Stomatal Density and Specific Leaf Area

Plant water status was calculated from the relative water content (RWC) of one full expanded leaf per plant (the mean value for five leaves in *E. paralias*). The leaves were excised in the field and transported to the laboratory in sealed zip-lock plastic bags. A whole leaf of *M. sinuata* and *C. soldanella*, a distal piece of leaf (*c.a.* 8 cm) of *P. maritimum*, and one disc (17 mm in diameter) taken from the distal part of a leaf of *E. maritimum* were used for measurements. The fresh mass of these materials was determined (M_f). Turgid mass (M_t) was measured after placing leaf material on filter paper saturated with distilled water in covered Petri dishes for 20 h at 4°C in the dark. The leaf material was blotted dry with tissue paper before the M_t was measured. The dry mass (M_d) was determined after oven drying at 75°C to constant weight. The RWC was calculated as $[(M_f - M_d) / (M_t - M_d)] \cdot 100$ (Peñuelas *et al.*, 2004; Chen *et al.*, 2009). Because of the small size of *E. paralias* leaves, the RWC was measured on whole leaves.

Stomatal densities were determined on one leaf per plant from impressions of the abaxial and adaxial mid-portions of leaf surfaces (in *E. paralias*, stomata are absent from the adaxial surface), which were obtained by applying a thin layer of clear nail polish. The dried nail-polish impressions were peeled from the leaves and mounted on a slide, and the stomata were counted in 100 × micrographs. One leaf was used per plant and three micrographs were captured per leaf (three leaves and one micrograph per leaf for *E. paralias*).

Two 500 × 500 μm frames per micrograph were used to calculate the stomatal density (stomata · cm⁻²). Polish imprints were not able to be obtained from *M. sinuata* because of the dense pubescence on the leaf surface.

Specific leaf area (SLA) was calculated for each individual plant on one fresh, fully expanded leaf of *M. sinuata* and *C. soldanella*, a distal piece of leaf (*c.a.* 8 cm length) for *P. maritimum*, one leaf disc (17 cm in diameter) for *E. maritimum* and the average of five leaves for *E. paralias*. The image of the projected leaf area was determined with a flatbed scanner (CanoScan LiDE 50, Canon Inc., Tokyo, Japan) and processed with a freely available public-domain image processing and analysis program (ImageJ software, National Institute of Health; Rasband, 1997-2011). The leaf material was then oven-dried at 75°C to constant weight and the mass was determined. The SLA was calculated as the one sided projected area of foliage per unit dry mass (cm² · g⁻¹ dry mass; Cornelissen *et al.*, 2003).

Leaf carbon and nitrogen content and isotopic composition

At all three sampling times, several leaves from each selected plant were cleaned of organic debris, dried at 75°C for over 5 days and then ball-milled to a homogenized fine powder to pass through a 40 μm mesh screen. The concentrations of total C and N (percentage of dry mass) and the molar ¹⁵N / ¹⁴N (δ¹⁵N) and ¹³C / ¹²C (δ¹³C) ratios were determined on subsamples (*c.a.* 1 - 2 mg dry wt), in an elemental analyzer (FlashEA 1112 Series) coupled to an isotope ratio mass spectrometer (ThermoFinnigan MAT253, Bremen, Germany). Carbon and nitrogen isotope ratios were expressed relative to the composition of a standard (Pee Dee Belemnite calcium carbonate for C, and air for N). The δ values (‰) were calculated as [(R_{sam} / R_{std}) - 1] · 1000, where R refers to the ¹³C / ¹²C or ¹⁵N / ¹⁴N ratio in the plant sample (R_{sam}) and standard (R_{std}), respectively. Polyethylene (IAEA-C6) and (NH₄)₂SO₄ (IAEA N1) were used as secondary international isotope standards for C and N,

respectively. The $\delta^{13}\text{C}$ values were transformed into carbon isotope discrimination values ($\Delta^{13}\text{C}$) by use of the following expression: $\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}) / (1 + \delta^{13}\text{C}_{\text{plant}})$ (Farquhar *et al.*, 1989), assuming a $\delta^{13}\text{C}$ air value of -8 ‰ on the PDB scale (Farquhar *et al.*, 1989). In C_3 plants, $\delta^{13}\text{C}$ values generally range between 15 ‰, for plants with higher water use efficiencies, and 25 ‰, for plants with lower water use efficiencies (Dawson *et al.*, 2002).

Soil measurements:

In June 2008, six soil samples (*c.a.* 25 cm in depth) were collected at a distance of 10 cm from the base of each species, and another six soil samples were collected from bare soil. The soil samples were transported in a cool box (4°C) to the laboratory, in hermetically sealed containers, and immediately weighed upon arrival. The samples were then dried to constant weight at 75°C and weighed, in order to estimate the soil water content ($\text{g water} \cdot \text{Kg}^{-1}$ dry soil). Subsamples of soils were used to determine the amount of inorganic phosphorous available to plants, by the Olsen's method (Olsen *et al.*, 1954). Dried subsamples (approximately 2 mg) of each sample were ground, sieved to < 2 mm and encapsulated in tin capsules. The samples were then analysed for carbon, nitrogen content via dry combustion at high temperature, in a CHNS elemental analyser (LECO CHNS-932; LECO Corp., St. Joseph, MI, USA). Soil salinity was estimated as electrical conductivity (EC), measured in a soil : water suspension (ratio 1 : 2.5), with an EC meter (524 Crison, Crison Instruments, Barcelona, Spain). Further subsamples (from a 1 : 2.5 dilution) were used to measure the pH in water and in KCl, with a pH-meter (pH-metro 507, Crison Instruments, Barcelona, Spain).

Statistical analysis:

Differences in leaf traits, %C, %N, $\Delta^{13}\text{C}$, $\delta^{15}\text{N}$, SLA, RWC and SD, were analysed by two-way Analysis of Variance (ANOVA), with R statistical

software; R Development Core Team, 2009). Species and sampling date were considered as fixed factors. The differences in soil samples for the variables considered were determined by one-way ANOVA. Prior to analyses, all variables were checked for normality and homoscedasticity required for Analysis of Variance. The C:N ratio and stomatal density were log-transformed prior to the analysis so that they met the underlying assumptions of the test. The slight deviations found for the other variables warranted the use of ANOVAs because this method is sufficiently robust to perform well when data do not deviate greatly from the underlying assumptions (Zar, 1984). Post-hoc pairwise comparisons between means were carried out by using Tukey HSD tests ("TukeyHSD" function of the package "stats" of R; R Development Core Team, 2009). Significance level for all tests was set at $\alpha \leq 0.05$.

Ordinary least squares (OLS) regressions (SMATR package, R statistical software; Warton *et al.*, 2007) were used to test for association between $\Delta^{13}\text{C}$ and %N, $\delta^{15}\text{N}$, SLA and RWC. A rotated principal component analysis (PCA) based on a correlation matrix of species by six leaf traits was performed with the aim of summarizing the main factors determining most of the variation in the data. The "prcomp" function of the "stats" package (R Development Core Team, 2009) was used to perform the PCA.

RESULTS

Leaf functional traits

Leaf nitrogen content

All species differed significantly in their leaf nitrogen contents, and the extent of these differences changed significantly over time (Table 1). There was a general trend in all species for nitrogen contents to be lower in June than in April, when the nitrogen content was highest (P always < 0.001 ; Fig. 1a), and

than in November. In *E. paralias*, *C. soldanella* and *P. maritimum* the nitrogen contents had fully recovered in November, whereas in *M. sinuata* the recovery was incomplete and in *E. maritimum* nitrogen content remained constant between June and November. At all times, the nitrogen content was highest in *P. maritimum*. On the other hand, the nitrogen content was lowest in *E. paralias* at all times, although the values were similar to those observed in *E. maritimum* in November.

Foliar carbon to nitrogen (C:N) ratio

Differences in C:N ratios of species varied significantly between sampling dates (Table 1; Fig. 1b). The C:N ratios in *E. paralias*, in which the C:N ratio was highest on all dates, and in *M. sinuata* and *E. maritimum*, were clearly higher in June than in April. In the two first species, the C:N ratios in November almost recovered to those initially observed in April, in *E. maritimum* the values were similar in June and November. The C:N ratios in *P. maritimum* and *C. soldanella* remained fairly constant over time.

Leaf nitrogen isotope composition $\delta^{15}\text{N}$

Species differences in leaf $\delta^{15}\text{N}$ varied significantly between the sampling dates (Table 1). There was no general pattern in the variation in $\delta^{15}\text{N}$ values of species between the sampling dates (Fig. 1c). The $\delta^{15}\text{N}$ values in *M. sinuata* and *P. maritimum* tended to decrease slightly between April and November, and in *E. paralias* and *E. maritimum* these values decreased significantly between April and June and then in November recovered or clearly exceeded (*E. maritimum*) the values observed in April. *Calystegia soldanella* was the only species in which the $\delta^{15}\text{N}$ values constantly increased between April and November. The $\delta^{15}\text{N}$ values were highest in *M. sinuata* and lowest in *E. paralias* for all dates throughout the period of study.

Table 1. Results of factorial analysis of variance for the effects of Species and Date on functional leaf traits (nitrogen content (%N), carbon to nitrogen ratio (C:N), nitrogen isotopic composition ($\delta^{15}\text{N}$), carbon isotopic discrimination ($\Delta^{13}\text{C}$), specific leaf area (SLA), relative water content (RWC), stomatal density (SD)). Significant differences ($P \leq 0.05$) are marked in bold and by *.

Factor(s)	%N			C:N			$\delta^{15}\text{N}$			$\Delta^{13}\text{C}$			SLA			RWC			SD		
	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F	df	SS	F
Species	4	61.23	125.04*	4	6038	255707*	4	329.38	45.15*	4	70	44.74*	4	59340	68753*	4	816	5.20*	3	130692	90.62*
Date	2	28.50	116.38*	2	1972	167014*	2	20.21	5.54*	2	23	28.73*	2	11532	26722*	2	3570	45.47*	2	1262	1.31a
Species \times Date	8	8.26	8.43*	8	876	18539*	8	46.17	3.16*	8	37	11.77*	8	10390	6019*	8	5029	16.01*	6	2372	0.82b
Residuals	150	18.36		150	885		150	273.58		150	59		150	32365		148	5810		123	59127	

Carbon isotope discrimination ($\Delta^{13}C$)

According to carbon isotope discrimination values, species-related differences in water use efficiency varied significantly between the different sampling dates (Table 1; Fig. 1d). In general, the extent of the differences among species was greater in November than in April or June, when the efficiencies were very similar in all species. Throughout the period of study, the efficiencies were highest in *E. paralias* and *P. maritimum* and lowest in *M. sinuata* and *C. soldanella*. The water use efficiency in *E. maritimum* was inconsistent, and it was highest in April and not significantly different from that in *E. paralias* or *P. maritimum*, but lowest in November and not different from that in *M. sinuata* or *C. soldanella*.

Specific leaf area (SLA)

The SLA differed significantly between species, and the extent of these differences varied significantly between the different sampling dates (Table 1; Fig. 1e). Except *P. maritimum*, in which SLA increased slightly (but not significantly) between April and November, the SLA values decreased in all species between April and June, especially in *E. paralias*, then recovered to or clearly exceeded the initial values (*C. soldanella*) in November. The SLA values were consistently lowest in *E. maritimum* and *P. maritimum*, with no significant differences between them, whereas the values were highest in *C. soldanella*.

Leaf relative water content (RWC)

The relative water contents were significantly different in the different species and the differences varied significantly between the different sampling dates (Table 1; Fig. 1f). The RWC values in *M. sinuata* differed most widely between the different sampling dates, and were lowest in this species (from among all species) in April and June, and highest in November. For this

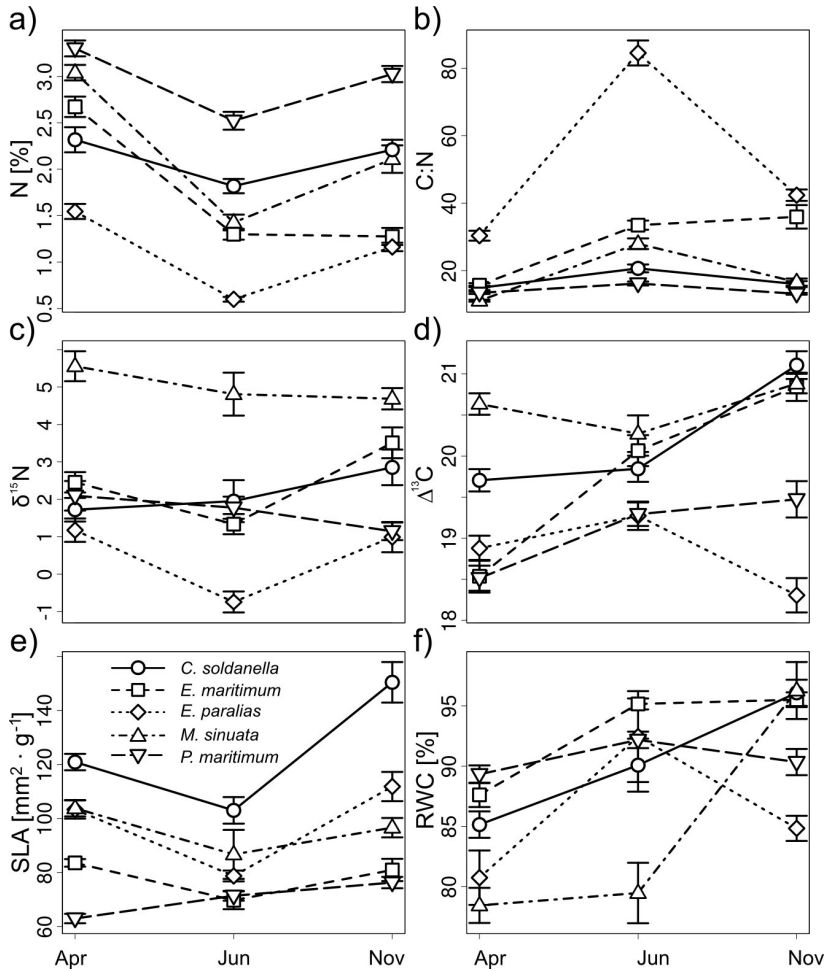


Fig. 1. Mean values (\pm S.E.) of leaf functional traits of the species under study at three different times (April, June and November): a) Nitrogen content (%N), b) carbon to nitrogen ratio (C:N), c) isotopic composition of nitrogen ($\delta^{15}\text{N}$), d) isotopic discrimination of carbon ($\Delta^{13}\text{C}$), e) specific leaf area (SLA, $\text{cm}^2 \cdot \text{g}^{-1}$) and f) relative water content (RWC).

species, the RWC in November differed significantly from those in April and June, but there was no difference between the values in the latter months. The RWC values also fluctuated greatly in *E. paralias*; the values increased

significantly between April and June, and then decreased slightly in November. The RWC values in the other species remained relatively constant over time, although they increased significantly between April and November in *C. soldanella*.

Stomatal density (SD)

The stomatal density differed significantly between species, but not between the different sampling dates (Table 1; Fig. 2). The stomatal density was lowest in *E. paralias*, followed by *P. maritimum*, *C. soldanella* and finally *E. maritimum*.

Relationships between leaf traits

The species differed significantly in the slope of the regression line for the relationship between $\Delta^{13}\text{C}$ and SLA (statistical test for common slope across species: 19.159, $P = 0.001$, Fig. 3a), although correlations were only significant for *C. soldanella* ($r^2 = 0.33$, $P < 0.001$) and *P. maritimum* ($r^2 = 0.37$, $P < 0.001$), and the positive slopes differed significantly ($P = 0.009$).

The slope of the regression line for the relationship between $\Delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was the same in the different species (statistical test: 8.676, $P = 0.077$, Fig 3b). This relationship was only significant for *C. soldanella* ($r^2 = 0.179$, $P = 0.013$), in which $\Delta^{13}\text{C}$ increased with increasing $\delta^{15}\text{N}$. Comparison of lines for the species revealed significant differences in the elevation for different species (Wald statistic = 76.563, $P < 0.001$), except for the comparisons between *C. soldanella* and *E. maritimum* ($P = 0.082$), *C. soldanella* and *M. sinuata* ($P = 0.111$), and *E. paralias* and *P. maritimum* ($P = 0.181$). The species also differed significantly in the shift along the common slope (Wald statistic = 170.96, $P < 0.001$), except in the comparisons between *C. soldanella* and *E. maritimum* ($P = 0.380$) and *C. soldanella* and *P. maritimum* ($P = 0.138$).

Species did not show a common slope for the relationship between $\Delta^{13}\text{C}$

and %N (statistical test: 22.625, $P = 0.001$, Fig. 3c). This relationship only was significant for *E. maritimum* ($r^2 = 0.508$, $P < 0.001$) and for *E. paralias* ($r^2 = 0.149$, $P = 0.022$), and the negative slopes for this relationship were significantly different between these species ($P = 0.052$).

For all species, the $\Delta^{13}\text{C}$ values tended to increase with the relative water content of leaves (Fig. 3d), but the species differed significantly in the slope of the regression line for this relationship (statistical test for common slope across species: 22.95, $P = 0.001$). However, this trend was only significant for *C. soldanella* ($r^2 = 0.359$, $P < 0.001$) and *E. maritimum* ($r^2 = 0.323$, $P = 0.001$).

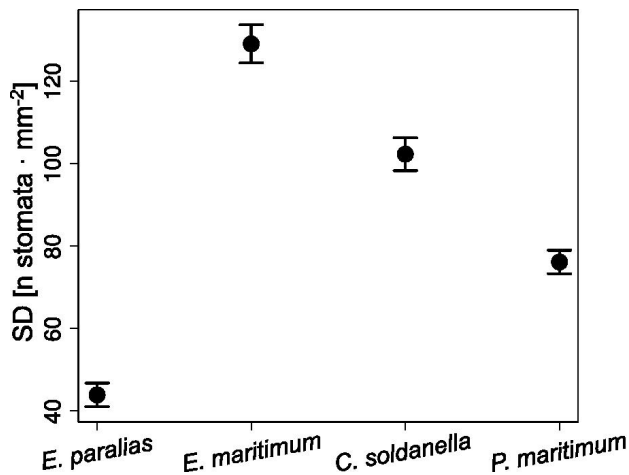


Fig. 2. Mean values (\pm S.E.) of stomatal density (SD, stomata · cm^{-2}) for the species under study.

Both species differed in the slope of this relationship ($P = 0.022$).

Soil variables

The species did not differ in the soil characteristics examined (Table 2), except for the pH in KCl, which was significantly higher in soils under *M.*

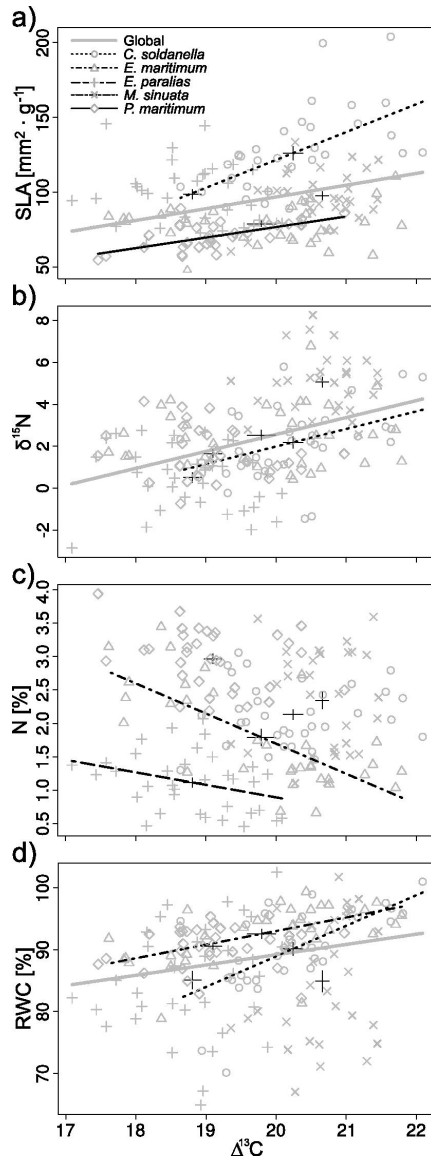


Fig. 3. Relationships between leaf carbon isotope discrimination ($\Delta^{13}\text{C}$) and a) specific leaf area, b) isotopic composition of nitrogen ($\delta^{15}\text{N}$), nitrogen content (%N) and relative water content (RWC) of the species under study. Crosses represent mean values (\pm S.E.) for the variables represented. Lines represent significant ($P \leq 0.05$) least-squares regressions for particular species or for all species.

sinuata, *E. paralias* and *P. maritimum* than in bare soils.

Principal component analysis

The three principal components extracted by PCA accounted for 76.4% of the total variance of the data matrix. The plant traits that loaded most heavily on the first factor, which accounted for 38% of the total variance, were those that expressed isotope ratios, with positive scores, and the percentage of carbon, with a negative score. The second factor, which explained 21.3% of the total variance, was positively correlated with the percentage of leaf nitrogen and negatively with RWC. Finally, the third principal component, which explained most of the residual variance (17.1%), was positively related to the SLA. The species were grouped in the three-dimensional space determined by the principal components (Fig. 4). The analysis revealed that the *E. paralias* data constituted the most segregated group, characterised by the highest leaf carbon content and WUE and lowest $\delta^{15}\text{N}$ values. On the other hand, the configuration of functional traits in *E. maritimum* and *P. maritimum* resulted in partially overlapped spatial distributions, although the SLA was lower and leaf nitrogen content and RWC were higher in *P. maritimum* than in *E. maritimum*.

DISCUSSION

The results of the study strongly suggest that the coexisting plants species under study have evolved specific morphological and physiological traits to survive in the harsh foredune environment. Most of the traits considered differed among the species and the differences changed significantly between the different sampling dates. This pattern of divergence may well determine differences in resource use, and thus the species may have differentiated their niches both in space and time. In species that are assumed to be competing, the limiting similarity process has often been used to explain patterns of trait

Table 2. Results of factorial analysis of variance for the effect of Species on the following soil variables: pH in water ($\text{pH}_{\text{H}_2\text{O}}$), pH in KCl (pH_{KCl}), total Carbon (C_{tot}) and Nitrogen content (N_{tot}), available Phosphorous (P_{av}), soil water content (SWC), soil salt content (Salinity).

	$\text{pH}_{\text{H}_2\text{O}}$		pH_{KCl}		C_{tot}		N_{tot}		P_{av}		Humidity		Salinity		
	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	SS	F	
Species	5	0.19	1.40	0.49	8.60*	151	0.72	$8.87 \cdot 10^{-4}$	1.83	1665	2.45*	3119	1.63	22.9	2.35
Residuals	30	0.81		0.34	1252		$2.91 \cdot 10^{-3}$		4075		11467		55.8		

divergence (Culver, 1970; Christiansen *et al.*, 1977). However, other trait-based studies have found that other processes may act simultaneously to promote species co-existence (Weiher *et al.*, 1998; Mouillot *et al.*, 2007; Andersen *et al.*, 2012).

The lack of significant differences in the soil characteristics of the species suggests that species did not select particular edaphic environments or greatly

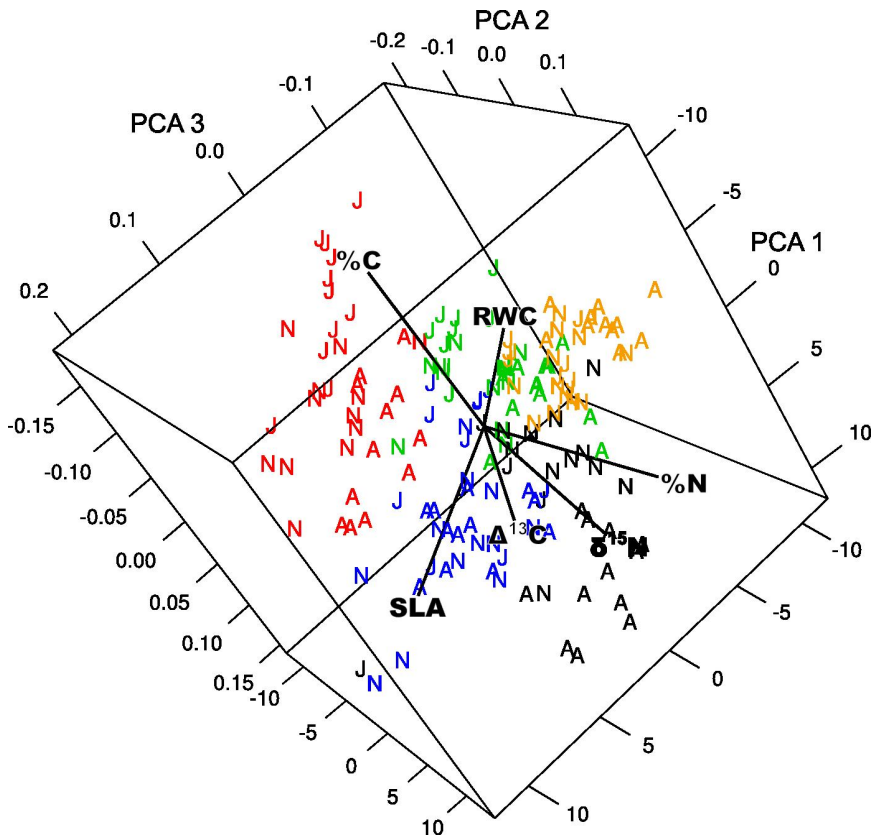


Fig. 4: Three-dimensional distribution of the studied species as a function of the three principal components resulting from principal component analysis based on a correlation matrix for leaf traits of the species under study. Colours represent the studied species: dark blue, *C. soldanella*; green, *E. maritimum*; red, *E. paralias*; black, *M. sinuata* and orange, *P. maritimum*. Letters represent the sampling date: (A) April, (J) June, and (N) November.

modify soil conditions. The pH in KCl of the soils under only three of the species was different from that of the bare soil. However, these findings do not necessarily indicate that the species had access to the same amount or type of soil resources. Although they coexist in a homogeneous area, they may exploit and use the resources differently in the soil horizons (resource partitioning across the soil profile). This aspect of plant niche was not directly investigated in the present study. However, the findings provide indirect evidence that support this assertion for two essential resources, water and nitrogen.

The enzyme Rubisco reacts more readily with $^{12}\text{CO}_2$ than it does with $^{13}\text{CO}_2$, and therefore carboxylation discriminates against the heavy isotope (Farquhar *et al.*, 1989). The extent of the discrimination depends on the concentration of CO_2 at the active site of Rubisco (C_i). Since C_i reflects the interaction between stomatal conductance and photosynthetic activity, carbon discrimination ($\Delta^{13}\text{C}$) has been related to processes such as leaf conductance, integrated WUE and photosynthetic capacity (Korner *et al.*, 1988; Farquhar *et al.*, 1989). In the present study, $\Delta^{13}\text{C}$ varied greatly among species, suggesting differences in how efficiently species use water. Species can coexist more readily if their water-use patterns differ as this reduces competition between them (Stubbs and Wilson, 2004). Although water use efficiency was always highest in *P. maritimum* and *E. paralias* and lowest in *M. sinuata* and *C. soldanella*, the relative efficiency of the species varied significantly between the different sampling dates. Therefore, the outcome of the competitive interactions for water may differ temporally, which may contribute significantly to the coexistence of the species. Temporal variation in rates of resource use may lead to greater similarity in the range of resources used by competitors (Abrams, 1983). The high WUE in *E. paralias* is consistent with the ability of this species to switch from C_3 to C_4 or CAM metabolism (Elhaak *et al.*, 1997; Daniela *et al.*, 2009). However, the generally low WUE in *C. soldanella* is somewhat surprising, in view of the recognised ability of this species to adopt a C_4 metabolism (Daniela *et al.*, 2009). The change in the

relative efficiency of the species in water use may be due to their ability to modify the metabolic route to fix CO₂ (temporal niche differentiation), in the case of *C. soldanella* and *E. paralias*, or to the different ability of species to access distinct sources of water that result from differences related to root distribution (spatial niche differentiation; Smedley *et al.*, 1991). In line with Rundel *et al.* (1999), we interpret the variability in $\Delta^{13}\text{C}$ in the species under study as evidence of different patterns in WUE and, therefore, of several strategies of adaptation to the dune environment. According to Fowler (1988), co-occurring species usually use resources in different ways, and therefore have different means of adapting to water scarcity. Similarly, Lo Gullo and Salleo (1988) showed that species sharing the same environment and similar morphological characteristics may adopt very different strategies to cope with water shortage, although other authors have found that species that differ greatly in morphological and taxonomic features may adopt similar physiological strategies to cope with drought, which suggests physiological convergence (Salleo *et al.*, 1990; Gouvra *et al.*, 2003).

Nitrogen availability is a critical factor limiting plant biomass over much of the biosphere (Gutschick, 1981; Vitousek *et al.*, 1991). Therefore, the way that plants acquire and use this resource is an important part of their survival strategy, which may influence community structure (Gutschick, 1981; Andersen *et al.*, 2012). In the present study, species differences in leaf nitrogen content changed significantly over time, although we did not find any significant differences between species in their soil nitrogen content. This finding is not consistent with the general observation that coexisting species are similar with respect to the concentrations of mineral nutrients in their leaves (see Grime (2006), and references therein), and suggests that the plants under study may display temporal differences in nitrogen demands, in their allocation to different structures and/or in their ability to take up soil nitrogen. However, in all species the leaf nitrogen contents were lowest in June. There are two plausible explanations for this. The plants were at the reproductive

stage in June (i.e. between May and September). At this stage, species may reallocate nitrogen from leaves to reproductive organs that act as strong sinks for the scarce nitrogen available (Karlsson, 1994). The soil water content is also lower in June than in April or November and it is well-known that soil water availability affects nutrient uptake through changes in diffusion and reduced root growth (Dunham *et al.*, 1976; Kuchenbuch *et al.*, 1986). The significant differences in the temporal patterns of variation in $\delta^{15}\text{N}$ in the species indicate that they differ in their strategies for nitrogen acquisition and use. The explanation for the variability in leaf $\delta^{15}\text{N}$ values is complicated by a variety of factors, such as discrimination during N uptake, mycorrhizal status, intra-plant isotope partitioning and mainly the specific N sources used by plants (e.g. NO_3^- and NH_4^+); these factors contribute to determining the $\delta^{15}\text{N}$ signature (Handley *et al.*, 1998; Kahmen *et al.*, 2008). As this study was not designed to address the variability in foliar $\delta^{15}\text{N}$ values, the results only provide integrated information about the species' strategies for nitrogen acquisition and use rather than about any specific process that explains foliar $\delta^{15}\text{N}$ variability. However, the results clearly show that species differ in their strategies for nitrogen acquisition and/or use. The highest $\delta^{15}\text{N}$ values observed throughout the period of study were in *M. sinuata*, and the respective lowest values were in *E. paralias*, so that the strategies are most different in these species, with the other species adopting intermediate strategies. According to Robinson *et al.* (2000), this could be interpreted in terms of stress tolerance, with the lower $\delta^{15}\text{N}$ values in the most stress-tolerant genotypes/species, irrespective of whether the stress was induced by drought or nitrogen starvation. Leaf $\delta^{15}\text{N}$ values may reflect the preference of a plant for a specific N source (NO_3^- or NH_4^+ ; Houlton *et al.*, 2007; Kahmen *et al.*, 2008), and thus one plausible interpretation for the variability in $\delta^{15}\text{N}$ values across species is that these may differ in the $\text{NO}_3^- : \text{NH}_4^+$ uptake ratio. However, there is no general consensus about this, with some studies reporting $\delta^{15}\text{N}$ decreased values with increased $\text{NO}_3^- : \text{NH}_4^+$ uptake ratio (Miller *et al.*,

2002; Falkengren-Grerup *et al.*, 2004), and others reporting the opposite (Kahmen *et al.*, 2008). In a study considering coexisting species, Andersen *et al.* (2012) found that leaf $\delta^{15}\text{N}$ (and also SLA) varied among species at all sites along a soil nutrient gradient, except for the site richest in nutrients. These authors suggested that competitive interactions at nutrient-poor sites, like the foredune system under study here, may have selected for species with divergent trait values, whereas trait values would converge to an optimal value in sites with higher nutrient availability. Other studies support this assertion, showing that leaf $\delta^{15}\text{N}$ varied among coexisting species in low nitrogen tundra habitats (McKane *et al.*, 2002; Ashton *et al.*, 2010), but community-wide convergence in tropical habitats where nitrogen is more readily available (Houlton *et al.*, 2007).

Negative relationships between leaf $\Delta^{13}\text{C}$ and nitrogen content have been reported for species growing under water-limited conditions (Retuerto *et al.*, 2000; Ma *et al.*, 2007; Ma *et al.*, 2012). In the present study, this relationship was only significant for *E. maritimum* and *E. paralias*, the negative slopes of which differed significantly. The photosynthetic capacity of leaves has been related to their nitrogen content (Evans, 1989); the more nutrients available to species, the faster their photosynthetic rate and the CO_2 demand, and consequently the lower the intercellular CO_2 concentration and carbon discrimination. Thus, the decrease in $\Delta^{13}\text{C}$ with increased nitrogen content suggests that in both species, especially in *E. maritimum* (for which the slope was more negative), the photosynthetic capacity is more important than stomatal diffusion in determining the $\Delta^{13}\text{C}$ values.

Specific leaf area may play a key role in plant functioning since it has been related to the plant's strategy for resource acquisition and use (Westoby, 1998; Vile *et al.*, 2005). SLA has also been considered to be the main factor determining growth variation, at least for herbaceous plants (Lambers *et al.*, 1992; Poorter *et al.*, 1998). The present results showed that SLA was generally lower in June than in April and November in all species, except *P. maritimum*.

The lower SLA and foliar nitrogen contents in June indicate that nitrogen limitation reduces SLA, as suggested by van Arendonk *et al.* (1997). This is also consistent with the significant negative slope of the regression line for the relationships between mass-based leaf nitrogen and leaf mass per area (SLA⁻¹), found in a global study considering many different growth forms, plant functional types and biomes (Wright *et al.*, 2004). However, other studies have shown that nitrogen limitation had no effect (van der Werf *et al.*, 1993) or that the SLA increased (Knops *et al.*, 2000). Lower SLA (i.e. thicker and/or denser leaves) has been associated with a higher photosynthetic capacity per unit leaf area (Dijkstra, 1989) and with a higher tolerance to drought (Givnish, 1979; Retuerto and Woodward, 1992; Salleo *et al.*, 2000). These findings are consistent with the significant positive relationships between SLA and $\Delta^{13}\text{C}$ in *P. maritimum* and *C. soldanella*, although the slope of the regression line was different for each species. Low SLA may also be a strategy for water conservation (Parkhurst *et al.*, 1972; Kramer *et al.*, 1995; Mulkey *et al.*, 1996) and for tolerating the high winds characteristic of dune environments (Woodward, 1983; Retuerto and Woodward, 1992). Low SLA, i.e. a large amount of photosynthetically active material per unit leaf area, may be particularly beneficial at the high levels of radiation experienced by plants during summer in the dune habitat. A lower SLA can be interpreted as an adaptive character for the more stressful summer conditions (June). We also observed less variation in this trait in June than in April or November, i.e. species were more similar to one another. It seems that under the more hostile conditions in summer, species tend to converge towards a more restricted range of values in SLA, which is consistent with the expectations from the environmental filtering theory. Although species differences in SLA varied significantly at the different sampling times, so that no single species had advantages in terms of SLA over the others over time, the SLA values were always highest in *C. soldanella*. If as predicted, climate change will increase the occurrence and severity of droughts (IPCC, 2004; Loik *et al.*, 2004), this

species might be more susceptible to suffering damage than the other coexisting species. On the whole, the species differences in SLA suggest niche differentiation in light capture and in the water retention ability of the plant. Stubbs and Wilson (2004) have convincingly demonstrated that co-occurring plant species in a New Zealand sand dune community were functionally spaced and showed low overlap in many characters, including SLA.

Relative water content has widely been accepted as a reproducible and meaningful indicator of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and osmotic adjustment, which is an effective mechanism of conserving cellular hydration under drought stress (Barrs *et al.*, 1962; Smart *et al.*, 1974). Differences in the RWC of the species under study depended on the time of year. In June, *M. sinuata*, which also had the lowest WUE (i.e. highest $\Delta^{13}\text{C}$), exhibited significantly lower RWC values than the other species. Considering the low values for both of these traits in April and June, we expect *M. sinuata* to have a competitive disadvantage, relative to the other species studied, under the predicted scenarios of higher temperatures and exacerbated water resources (IPCC, 2004). Summer conditions appear to impose restrictions to the variability in RWC, as observed for SLA and $\Delta^{13}\text{C}$. However, even in June, the RWC of all the species were much higher than the values at which wilting may appear (Ludlow, 1989) and plants were able to maintain their potential for carbon acquisition and growth.

The only positive and significant relationship between $\Delta^{13}\text{C}$ and foliar RWC was in *C. soldanella* and *E. maritimum*. Thus, in both species $\Delta^{13}\text{C}$ is very sensitive to changes in leaf water content and the plants can adjust the WUE accordingly. This ability is probably an important determinant of plant fitness in arid environments such as the foredune system under study. The stomatal densities were also highest in *C. soldanella* and *E. maritimum*. Since $\Delta^{13}\text{C}$ is a very good indicator of the leaf intercellular concentration of CO_2 (C_i), and this reflects the balance between consumption and supply rates of

CO₂ by photosynthetic activities and stomatal diffusion, the results clearly suggest inter-species differences in the extent to which stomata limit photosynthetic gas exchange rates. The significantly greater slope for *C. soldanella* than for *E. maritimum*, means that for the same RWC, the water-use efficiency is lower in *C. soldanella*.

Stomatal density may play a main role in plant responses to environmental conditions (Hetherington and Woodward, 2003) because plants may control water loss and CO₂ uptake by varying stomatal density when they produce new leaves. Stomatal density has been found to be positively correlated to stomatal conductance, net CO₂ assimilation rate and water use efficiency, revealing that increased stomatal density may enhance plant adaptations to drought (Spence *et al.*, 1986; Retuerto *et al.*, 1993; Xu *et al.*, 2008). In view of these findings, the significant differences in the stomatal density suggest that the species under study may differ in their vulnerability to drought. Considering that stomatal densities were highest in *E. maritimum* and *C. soldanella*, these species should be the least vulnerable to drought. The WUE was also highest in these species, as indicated by the $\Delta^{13}\text{C}$ values, which supports the previously reported positive correlations between stomatal density and $\Delta^{13}\text{C}$, both of which are closely linked to carbon fixation and water status in C₃ plants (van de Water *et al.*, 1994).

Principal component analysis summarized most of the variability in the dispersion matrix of descriptors in a few dimensions. The spatial segregation of the species in the 3D-plot generated by the PCA supports the view that each species may be characterized by a specific configuration of functional traits that define different strategies in resource use. In fact, each species could be characterized by a different set of values for the relevant leaf traits with the highest loadings on the three main components. Only the distributions of *P. maritimum* and *E. maritimum*, which occupied the closest positions, partly overlapped.

On the whole, the results showed a functional spacing of coexisting species, which has been considered as evidence that limiting similarity processes may operate within a community (Ranta *et al.*, 1981; Armbruster *et al.*, 1994; Stubbs and Wilson, 2004). However, the spatial and temporal heterogeneity generated by disturbance factors operating in coastal dune systems (disturbance filter, *sensu* Grime, 2006) may also explain the divergence in traits and niche differentiation observed in the coexisting species. Grime (2006) stated that disturbance drives trait variation and species co-existence at a local scale more strongly than competition. Disturbance may generate constantly changing scenarios for which species cannot evolve an optimal strategy since this would change with environmental conditions. This would favour multiple suboptimal strategies, the relative performance of which would depend on environmental conditions.

In conclusion, the present study demonstrated that the species coexisting in a foredune system differed widely in several important functional traits related to the use of essential resources such as water, nitrogen and light. This supports the idea that species may coexist more readily if they reduce competition by differing in their resource-use strategies. We also demonstrated temporal species-specific effects (temporal niche differentiation) on resource use, which implies that the outcome of competition will vary temporally to favour species coexistence. Consequently, and somewhat contrary to our expectation that stress factors in the hostile foredune system would severely constrain the range of physiological responses to a limited number of possibilities (functional convergence), the findings provide evidence that limiting similarity processes may be more important in structuring the community under study. However, the results are also consistent with the idea of a community in a state of non-equilibrium, in which competitive equilibrium may be prevented by a wide range of factors, including fluctuations in physical factors and random factors. Although we found that the coexisting species differed in traits that may affect their vulnerability to

environmental changes, the contribution of both deterministic and chance/randomness process to structuring the community makes it difficult to predict how coexistence of the species would be affected in the future. Finally, findings indicating that the coexisting species occupy quite different functional space suggest a high degree of functional diversity and complexity in this coastal system. Potential changes in such vulnerable systems threatened by factors such as urban expansion, species invasion or a rise in the sea level as a result of climate change may remove processes that are unique to these systems and that have important effects on ecosystem functioning and the services that they provided.

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Chapter 2.

One site but many types: Evidences of functional trait divergence in leaves of five perennial herbs coexisting in a coastal dune environment.

SHORT TITLE: Functional divergence in coexisting dune herbs.

KEYWORDS: Coastal sand dunes; Coexisting species; Environmental filtering; Functional similarity; Leaf reflectance; Photochemical efficiency.

ABSTRACT

Ecological theory suggests that, in harsh environments, major abiotic stress factors may act as environmental filters, thereby reducing the range of possible plant strategies through environmental trait selection. This would lead to functional similarity of species coexisting in hostile environments. To test this prediction, we evaluated six functional leaf traits, at three different times of year, in five species coexisting in a coastal dune ecosystem. The functional

traits examined were associated with water status and light interception and use, two of the most limiting stress factors in dune systems. Species differed in traits associated with light absorption, namely chlorophyll content, the vigour index NDVI, and the proportion of the light absorbed that is used in photochemistry (as expressed by the effective quantum yield of photosystem II and the photochemical reflectance index). For most of the traits, the relative performance of species depended significantly on time. This research revealed a significant divergence in functional traits of coexisting species, which does not conform to findings in other harsh environments where species tend to functional convergence. The data provide experimental support for the hypothesis that there is no single combination of traits for a given environment, but that alternative functional designs of similar fitness may evolve in the same environment as a result of complex interactions and trade-offs among traits. This suggests that factors claimed to promote divergence, such as limiting similarity and disturbance processes, may play an important role in structuring the dune community under study. The high variability in functional traits suggests a significant degree of functional diversity and highlights the importance of preserving the species composition of threatened coastal dune habitats in order to preserve distinctive functional processes that may be unique to the systems.

INTRODUCTION

The study of how plants can survive and grow in sites characterized by severely unfavourable environmental conditions has been central to physiological ecology (Crawford, 1989). Understanding how plants deal with extreme conditions provides insights into why certain plant traits are selected under such hostile environments. Coastal sand dune systems (especially foredunes) are one example of this type of environment where plant function is severely limited by environmental restrictions and mere survival is a

challenge. Some of the limiting stress factors and types of disturbance that plants must endure in dune systems include the following: high levels of airborne and soil salinity (Barbour, 1978; de Jong, 1979; Hesp, 1991; Maun, 1994; Cheplick *et al.*, 1999; Cheplick *et al.*, 2002; Ogura and Yura, 2008) low availability of water (de Jong, 1979; Gagne and Houle, 2002; Ripley *et al.*, 2004) and nutrients (Kachi *et al.*, 1983; Maun, 1994; Houle, 1997); sand blasting, burial and flooding (Disraeli, 1984; Maun, 1994; Maun, 1998; Yura *et al.*, 2006; Ogura and Yura, 2008; Kim *et al.*, 2009); and high intensities of incident and reflected light (Chapman, 1976; Barbour *et al.*, 1985). In addition, some of the stress factors and disturbances that operate in coastal environments vary widely in frequency and intensity and are therefore unpredictable (Maun, 1998; Gagne and Houle, 2002; Alpert *et al.*, 2002; Orr *et al.*, 2005; Dech *et al.*, 2006; Maun, 2009; Reed *et al.*, 2010). This imposes further restrictions on the survival of plant species in hostile environments. Plant species have evolved numerous adaptations to complex combinations of environmental factors in order to survive in coastal dune environments. Survival strategies in plants involve adjustments at many levels: molecular (Khedr *et al.*, 2003); cellular, (Blackman *et al.*, 2005; Pierce *et al.*, 2005); physiological (Blackman *et al.*, 2005; Daniela *et al.*, 2009); morphological (Hegazy *et al.*, 1998; Voronkova *et al.*, 2008; Daniela *et al.*, 2009); and phenological and community (Knevel *et al.*, 2004; Armas *et al.*, 2009).

Ecological theory suggests that, in harsh environments, major abiotic stress factors may act as environmental filters, thereby reducing the range of possible plant strategies through environmental trait selection (Abrams, 1983; Webb *et al.*, 2002; Cornwell *et al.*, 2006; Grime, 2006; Mouillot *et al.*, 2007). Ecological theory also predicts trait convergence in species that coexist in hostile environments (as result of the process of environmental filtering), which would lead to functional similarity among species (Keddy, 1992; Blackman *et al.*, 2005; Jacobsen *et al.*, 2008; Gallego-Fernández *et al.*, 2011). Despite the severe restrictions to plant survival imposed by coastal dune

environments, one outstanding feature of these environments is the high ecological diversity in terms of species composition (van der Maarel, 2003; Acosta *et al.*, 2009). According to Ehrenfeld (1990), the large diversity of adaptations in coastal dune plants is suggestive of a diversity of microhabitats on a small spatial scale as well as diverse mechanisms of adaptation. In the general theory of competition and coexistence in variable environments, Chesson *et al.* (2004) distinguishes two main mechanisms that contribute to maintaining such diversity: a) the storage effect, and b) the relative nonlinearity of competition. The storage effect explains species coexistence in variable environments by the storage of resources in organs when conditions are favourable and competition is low, which enables species to compensate for periods of intense competition. The relative nonlinearity of competition suggests that the relative performance of species in resource acquisition and their competitive ability depend on time, which minimizes the competition and contributes to the coexistence of species. In line with studies that report a large variety of adaptations in dune plants, several authors have observed significant functional spacing of coexisting species, strongly suggesting that alternative mechanisms to environmental filtering may be important in community assemblages. For example, trait divergence and niche differentiation in coexisting species have been considered as evidence that limiting similarity processes, induced by competition (Ranta *et al.*, 1981; Armbruster *et al.*, 1994; Stubbs and Wilson, 2004) or by disturbance factors (disturbance filter, *sensu* Grime, 2006), operate within communities, thus allowing species to coexist. Other processes such as facilitation and mutualism may lead to either divergence or convergence of traits (Valiente-Banuet *et al.*, 2007; Sargent *et al.*, 2008). Species in communities will be selected according to their functional traits, as a result of the dynamic balance among the different forces structuring the communities.

In the context of the debate on the relative importance of the different mechanisms that determine community assemblages, this study aims to

examine some aspects of functional diversity in traits associated with photosynthetic efficiency, which may directly affect the niches of five species coexisting in a coastal foredune system. The harshness of foredune environments suggests that environmental filtering processes that promote trait convergence may predominate at the assemblage level, leading to functional similarity of species in such habitats (Cornwell *et al.*, 2006; de Bello *et al.*, 2009). However, other processes may act simultaneously in the opposite direction, leading to exclusion of functionally similar species, and thus to species niche partitioning by trait divergence (MacArthur *et al.*, 1967; Stubbs and Wilson, 2004). Assuming the latter, and considering the extensive range of morphologies and phenologies observed in the foredune species under study, we expect significant species differentiation in functional leaf traits associated with water status and light interception and use. To test this hypothesis, we evaluated six functional leaf traits, at three different times of year, in two seasons: at the beginning and at the end of summer (presumably the most critical period), and in winter. Differences in these physiological parameters may explain spatial and temporal niche segregation in the species under study and the high level of functional diversity with respect to photosynthetic performance. A greater understanding of the physiological functioning and functional diversity of species is imperative to successful management of coastal sand dune systems, which are seriously threatened as a result of persistent pressures associated with urban expansion, recreational development, invasion by non-native plants and the rising sea level due to climatic change (Maun, 2009). Knowledge of the physiological functioning of foredune species is also critical to understanding their effects on ecosystem-level processes such as resource fluxes, stability and invasibility (Tilman *et al.*, 1997; Diaz and Cabido, 2001; Eviner *et al.*, 2003; Sandquist *et al.*, 2007). Finally, knowledge of species differences in physiological functional traits is relevant in the context of on-going environmental changes, since habitat modifications can alter functional diversity and functional composition and

have a predictably large impact on ecosystem processes.

MATERIALS AND METHODS

Study species

For the study, we selected five species of perennial herbs with contrasting morphological leaf traits and life-forms, from among the most widespread and dominant species in coastal dune environments in Galicia (NW Spain). *Matthiola sinuata* (L.) R. Br. (Brassicaceae) is a hemicryptophyte with a basal rosette of narrow highly pubescent pale grey-green leaves. The plants grow up to 40 cm in height and the leaves up to 10 cm in length. *Euphorbia paralias* L. (Euphorbiaceae) is a chamaephyte with both fertile and sterile branches growing from a woody base and with closely overlapping leaves. The leaves, of mean size 20 mm, are thick, ovate to elliptic; white latex emanates when the leaves are excised. The stems branch from a woody base. *Eryngium maritimum* L. (Umbelliferae) is a hemicryptophyte with three-lobed folded blue-green leaves. The leaves are stiff, leathery and spiny. *Calystegia soldanella* (L.) R. Br. (Convolvulaceae) is a hemicryptophyte with fleshy, kidney or heart shaped leaves. The large trumpet-shaped flowers are pink to purple-pink in colour, with lighter stripes. *Pancratium maritimum* L. (Amaryllidaceae) is a cryptophyte with a bulbous stem from which a long glaucous neck grows, ending in thick, elongated, linear-shaped leaves of up to 40 cm in length. All of these species are C₃ plants, although under certain environmental conditions *C. soldanella* and *E. paralias* can switch to C₄ metabolism, and *E. paralias* can switch to CAM metabolism (Elhaak *et al.*, 1997).

Experimental protocol

A homogeneous flat area of about 220 m² was delimited in the foredune of

the Lariño coastal dune system (42°46'00"N, 9°06'58"W; Galicia, NW of Spain), where all the above species co-occurred. A number of individuals of each species were selected at random and tagged, for measurement of leaf spectral reflectance and chlorophyll fluorescence parameters in different seasons (winter and summer).

Leaf spectral reflectance

The reflectance spectra of leaves were measured in nine randomly selected individuals per species, in summer (July and September 2005) and winter (March 2009). In September 2005, measurements were made on the same plants that had been selected and tagged in July 2005. The September data were used for seasonal comparison (summer - winter). The measurements were made on the adaxial side of one fully expanded leaf per plant, with a portable spectrometer (Unispec, PP Systems, Haverhill, MA, USA). A reflective white standard (Spectralon Reflectance Standard, Labsphere, North Sutton, NH, USA) was used to obtain the reflectance spectra (wavelength range from 306 to 1136 nm), by dividing the spectral radiance of the leaf by the radiance of the standard reference. The chlorophyll index was calculated (CHL) from the reflectance data, as the R_{750} / R_{550} ratio (Lichtenthaler *et al.*, 1996; Gitelson and Merzlyak, 1997). R_{750} and R_{550} represent the reflectance at 750 and 550 nm, in the near infrared (NIR) and visible (green) range, respectively. R_{750} is relatively insensitive to variations in the chlorophyll content of leaves (Lichtenthaler *et al.*, 1996), whereas R_{550} is sensitive to such changes. For several species, this index has been shown to be positively and linearly correlated with the chlorophyll content of leaves determined spectrophotometrically in a 100% acetone extract, with an estimation error of within $4.9 \mu\text{g} \cdot \text{cm}^{-2}$ (Gitelson and Merzlyak, 1997). The normalized difference vegetation index (NDVI) was calculated as $(R_{800} - R_{680}) / (R_{800} + R_{680})$ (Peñuelas *et al.*, 1994; Gamon *et al.*, 1995). This index, which is correlated with leaf chlorophyll content, photosynthetic efficiency, foliar nitrogen,

phosphorous and potassium content (Peñuelas *et al.*, 1993; Gitelson *et al.*, 1994; Gamon *et al.*, 1995; Albayrak *et al.*, 2011), has been related to the “greenness” or general vigour of vegetation. It uses the reflectance at 680 nm, which is sensitive to variations in chlorophyll concentration, and at 800 nm, which is sensitive to leaf water content. The water index (WI) was computed as the R_{900} / R_{970} ratio (Peñuelas *et al.*, 1997; Peñuelas and Inoue, 1999), where the reflectance at 970 nm reflects variations in leaf water content, and the reflectance at 900 nm is used as a reference since although there is no water absorption at this wavelength, it is affected in the same way by the sample structure (Peñuelas *et al.*, 1997). The WI is closely correlated with stomatal conductance (gs; Serrano *et al.*, 2010) and with relative water content (RWC; a gravimetric index; Peñuelas and Inoue, 1999). The WI was corrected for the effect of NDVI, because the WI / NDVI ratio is more closely correlated with leaf water content (Peñuelas *et al.*, 1997; Peñuelas and Inoue, 1999). The photochemical reflectance index (PRI), which is a reflectance-based photosynthetic parameter, was also calculated as $(R_{531} - R_{570}) / (R_{570} + R_{531})$ (Gamon *et al.*, 1992; Peñuelas *et al.*, 1995). R_{531} is sensitive to changes in the epoxidation state (EPS) of the xanthophyll cycle, transthylakoid pH gradient formation and conformational changes in chloroplasts (Demmig-Adams, 1990), whereas R_{570} is insensitive to changes in these processes and is used as a reference (Gamon *et al.*, 1992). The PRI is directly correlated with radiation use efficiency (RUE; mol CO₂ · mol⁻¹ photons) and photosynthetic rate, and inversely correlated with the dissipation of excess radiation energy as heat (Gamon *et al.*, 1992; Peñuelas *et al.*, 1994; Peñuelas *et al.*, 1995; Filella *et al.*, 1996; Gamon *et al.*, 1997).

Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured with a portable pulse amplitude modulated fluorometer (Photosynthesis Yield Analyser Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany). Pulses of measuring light and

saturating light ($> 4000 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 0.8 s pulse length, actinic white light) were applied through a fibre-optic probe (active cross section = 6 mm) at an angle of 60° relative to the sample and 10 mm from the leaf. The effective quantum yield of photosystem II (ΦPSII) was calculated as $(F'_m - F_t) / F'_m$ (Genty *et al.*, 1989), where F'_m is the maximum intensity of fluorescence emitted by an illuminated sample after a pulse of saturating light, and F_t is the basal fluorescence emission of the leaf at a given photosynthetic photon flux density (PPFD; Maxwell and Johnson, 2000; Roháček, 2002). This index, which measures the proportion of the light absorbed by the chlorophyll used in photochemistry, was measured on the same dates and in the same plants and leaves selected to register leaf spectral reflectance. An Arabidopsis Leaf Clip Holder 2060-B (Heinz Walz GmbH, Effeltrich, Germany) coupled to the fluorometer probe was used under a PPFD of $900 (\pm 50) \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Several studies have found that this parameter is inversely correlated with the dissipation of excess radiation energy as fluorescence and directly correlated with radiation use efficiency (Genty *et al.*, 1989; Maxwell and Johnson, 2000; Roháček, 2002). On the same dates and in six of the nine plants selected to measure ΦPSII , the maximum quantum yield of photosystem II (F_v / F_m) was determined as $(F_m - F_0) / F_m$, where F_m and F_0 are the maximum and the basal fluorescence emission, respectively, in dark adapted samples, with all reaction centres of photosystem II fully open (i.e. all primary acceptors oxidized). Leaves were dark adapted for 30 min before measuring the F_v / F_m by use of Dark Leaf Clips (Heinz Walz GmbH, Effeltrich, Germany). This length of time has been considered sufficient to ensure that all reaction centres of photosystem II are open (Roiloa *et al.*, 2005). The F_v / F_m ratio estimates the efficiency by which the excitation energy is captured by open photosystem II reaction centres and represents the fraction of incident photon energy that is processed photochemically (Krause *et al.*, 1991; Mohammed *et al.*, 1995). A decrease in F_v / F_m values to below 0.8 has been considered symptomatic of stress-dependent photoinhibition (Bjorkman

and Demmig, 1987; Long *et al.*, 1994; Maxwell and Johnson, 2000).

Data analysis

Within summer differences (July compared with September) in functional traits were analyzed by repeated measures analysis of variance (rANOVA), in order to consider the dependence between observations within subjects. Species was considered as a fixed between-subject factor and Month as a within-subject factor. The effect of Season (summer compared with winter) on species differences was estimated from the Species \times Season interaction term in a two-way ANOVA, with Species and Season as fixed factors. All data were previously tested for normality and homogeneity of variance. The chlorophyll index (CHL) and leaf water content index (WI / NDVI) were \log_{10} -transformed in both the within summer and between season comparisons. The maximum quantum yield of photosystem II (F_v / F_m) for the within summer analysis was also \log_{10} -transformed. When ANOVA detected significant effects of factors, the Tukey HSD test was used to check the pairwise differences among levels of single factors. The significance threshold considered for all statistical analyses was $\alpha = 0.05$, and the analyses were computed with SPSS v17.0 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

Leaf traits associated with light absorption

Within summer analysis

Species differed significantly in chlorophyll content (Table 1). The chlorophyll contents were highest in *C. soldanella* (P always < 0.001 , for pairwise comparisons), followed by *P. maritimum* (P always < 0.001) and *M. sinuata* (P always ≤ 0.048). The chlorophyll contents were lowest in *E.*

maritimum and *E. paralias* (P always ≤ 0.048), with non-significant differences between these species ($P = 0.734$). In general terms, leaf chlorophyll content decreased between July and September (Table 1), specifically in *P. maritimum*, *M. sinuata* and *E. paralias*, although the Species \times Time effect was not statistically significant (Table 1; Fig. 1a). The vigour of the vegetation, estimated by the NDVI index, differed significantly among species (Table 1). The values were highest in *C. soldanella* (P always < 0.001), followed by *P. maritimum*, *E. paralias* and *M. sinuata*. The NDVI values were lowest in *E. maritimum* (P always < 0.001). The NDVI values in *E. paralias* and *M. sinuata* differed significantly from those in the other species (P always ≤ 0.026), but not from each other ($P = 0.274$). Except in *E. maritimum*, the NDVI values decreased between July and September, with a statistically significant Species by Time effect (Table 1; Fig. 1b).

Seasonal analysis

For all the variables measured, the relative physiological performance of species varied significantly with season (Table 2). The species were similar as regards the chlorophyll (CHL) and vigour (NDVI) indices (Fig. 1a and b). The winter values of these indices were quite similar for all species, and they were significantly higher than summer values, except in *C. soldanella*, for which the summer values were significantly higher than those for the other species and close to the winter values.

Leaf traits associated with light processing

Within summer analysis

The values of photochemical reflectance index were significantly lower in *E. paralias* than in *P. maritimum* and *E. maritimum* (maximum $P \leq 0.033$, for pairwise comparisons). However, species differences in PRI depended significantly on Month (Table 1; Fig. 1d). The PRI values in *M. sinuata* and *P.*

Table 1. Results of the repeated measures ANOVA for the leaf functional traits: Chlorophyll content (CHL, \log_{10} -transformed), vigour index (NDVI), water content (WI / NDVI, \log_{10} -transformed), photochemical reflectance index (PRI), and effective quantum yield of photosystem II (Φ PSII). Month was included as within subject (dependent) factor, and Species as fixed factor. *P*-values < 0.05 are shown in bold type.

	<i>df</i>	CHL			NDVI			WI/NDVI			PRI			Φ PSII		
		SS	<i>F</i>	<i>P</i>	SS	<i>F</i>	<i>P</i>	SS	<i>F</i>	<i>P</i>	SS	<i>F</i>	<i>P</i>	SS	<i>F</i>	<i>P</i>
Between subjects																
Species	4	$6.81 \cdot 10^{-1}$	86.38	<0.001	1.54	98.13	<0.001	1.35	32.2	<0.001	$7.59 \cdot 10^{-3}$	4.44	0.004	$4.71 \cdot 10^{-1}$	19.82	<0.001
Error	45	$8.88 \cdot 10^{-2}$			$1.76 \cdot 10^{-1}$			$4.72 \cdot 10^{-1}$			$1.92 \cdot 10^{-2}$			$2.67 \cdot 10^{-1}$		
Within subjects																
Month	1	$2.43 \cdot 10^{-2}$	15.36	<0.001	$1.56 \cdot 10^{-1}$	78.83	<0.001	$3.32 \cdot 10^{-2}$	8.87	0.005	$4.3 \cdot 10^{-6}$	0.01	0.91	$1.43 \cdot 10^{-3}$	0.25	0.617
Species x Month	4	$1.43 \cdot 10^{-2}$	2.27	0.076	$9.83 \cdot 10^{-2}$	12.40	<0.001	$9.49 \cdot 10^{-2}$	6.33	<0.001	$5.8 \cdot 10^{-3}$	4.36	0.005	$4.09 \cdot 10^{-2}$	1.81	0.144
Error(Month)	45	$7.12 \cdot 10^{-2}$			$8.92 \cdot 10^{-2}$			$1.68 \cdot 10^{-1}$			$1.49 \cdot 10^{-2}$			$2.54 \cdot 10^{-1}$		

maritimum did not vary between July and September, whereas the PRI values in *E. maritimum* and *E. paralias* decreased between July and September and increased in *C. soldanella* within this period. Species differed significantly in the proportion of light absorbed by chlorophyll that is used photochemically, as expressed by ΦPSII (Table 1; Fig. 1e), although no Month or Month \times Species effect was detected. The values of the effective quantum yield of photosystem II were lowest in *E. paralias* (P always < 0.001 , for pair-wise comparisons). No Species or Month effects on the maximum quantum yield of photosystem II were detected (F_v / F_m ; P always ≥ 0.113).

Seasonal analysis

The relative performance of species, as expressed by variables related to photochemical efficiencies (PRI and ΦPSII), depended significantly on Season. Both the PRI and ΦPSII values were much higher in summer than in winter, except in *M. sinuata* and *E. paralias*, in which the values were similar in both seasons (Table 2; Fig. 1d and e). In both summer and winter, the ΦPSII values were highest in *M. sinuata* and lowest in *E. paralias*. The ranking of species in terms of PRI values clearly depended on Season, especially for *C. soldanella* and *E. maritimum*, in which the PRI values were lowest in winter, but among the highest in summer. The values of maximum quantum yield of photosystem II were lower in winter than in summer in *E. maritimum*, *P. maritimum* and especially *C. soldanella* (in which the F_v / F_m value was significantly lower in winter; Table 2; Fig. 1f).

Leaf traits associated with water content

Within summer analysis

Differences in the leaf water content of species, as estimated by the WI / NDVI ratio, depended significantly on Time (Table 1; Fig. 1c). Leaf water content increased significantly between July and September in *E. paralias*, *M.*

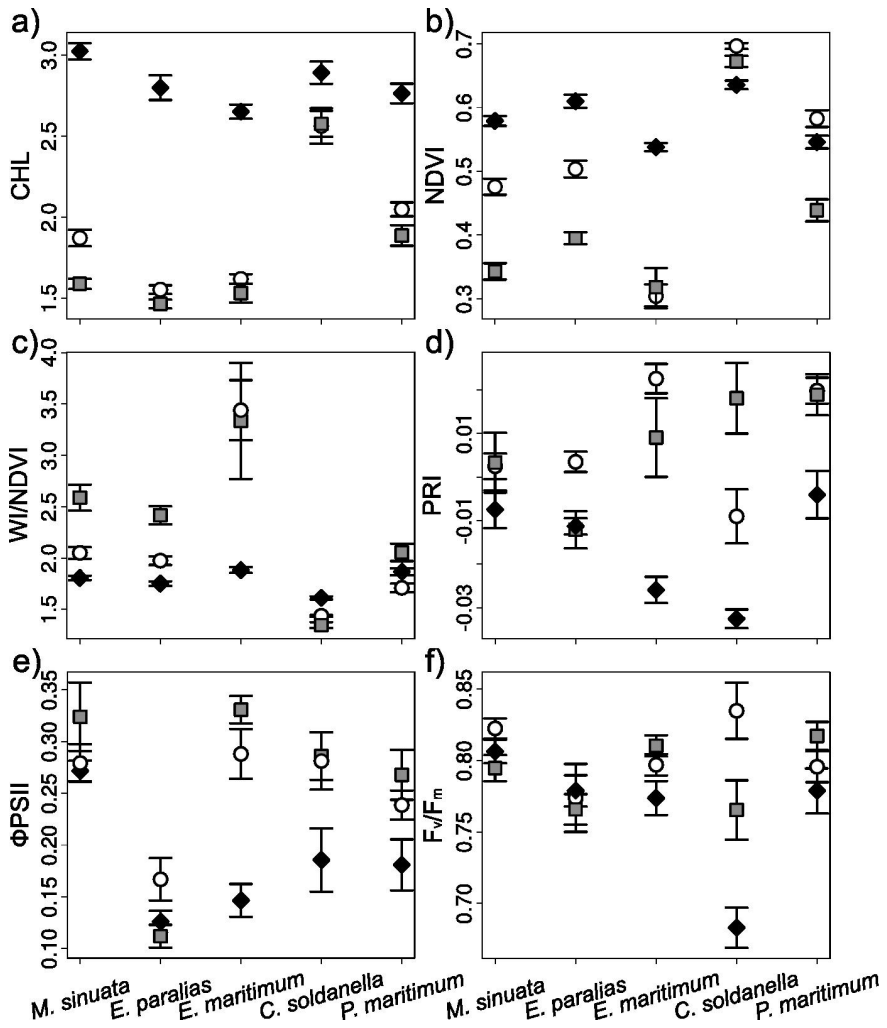


Fig. 1. Mean values (\pm S.E.; $n=9$) of leaf functional traits of the species under study, at three different times in two seasons, summer (July, \circ ; September, \blacksquare) and winter (March, \blacklozenge): a) leaf chlorophyll content (CHL index), b) vigour index (NDVI), c) leaf water content (WI / NDVI), d) photochemical reflectance index (PRI), e) effective quantum yield of photosystem II (Φ PSII), and f) maximum quantum yield of photosystem II (F_v / F_m).

Table 2. Results of the ANOVA for the leaf functional traits: Chlorophyll content (CHL, \log_{10} -transformed), vigour index (NDVI), water content (WI / NDVI, \log_{10} -transformed), photochemical efficiency by reflectance (PRI), effective quantum yield of photosystem II (Φ PSII) and maximum quantum yield of photosystem II (F_v / F_m). Species and Season were included as fixed factors. P -values < 0.05 are shown in bold type.

	CHL			NDVI			WI/NDVI			PRI			Φ PSII			Fv/Fm			
	df	SS	F	P	SS	F	P	SS	F	P	SS	F	P	SS	F	P	SS	F	P
Species	4	0.206	52.06	<0.001	0.556	156.74	<0.001	0.446	72.44	<0.001	0.006	5.16	0.001	0.326	15.00	<0.001	0.045	8.73	<0.001
Season	1	1.003	1015.31	<0.001	0.558	629.73	<0.001	0.217	140.92	<0.001	0.009	34.06	<0.001	0.144	26.53	<0.001	0.029	22.53	<0.001
Species x Season	4	0.164	41.55	<0.001	0.252	71.01	<0.001	0.228	37.06	<0.001	0.007	6.16	<0.001	0.093	4.27	0.003	0.037	7.06	<0.001
Error	80	0.079			0.071			0.123			0.022			0.434			0.104		

sinuata and *P. maritimum*, but did not vary in *C. soldanella* and *E. maritimum*. In both July and September, the leaf water content was highest in *E. maritimum* (P always ≤ 0.001 , for pairwise comparisons) and lowest in *C. soldanella* (P always ≤ 0.003).

Seasonal analysis

The trend in leaf water content values was to some extent the opposite of the trends in chlorophyll content and vigour indices (Table 2; Fig. 1c), with significantly lower values in winter than in summer (which were more variable than winter ones), except in *C. soldanella*.

For the species under study, the characteristic combinations of functional leaf traits or species strategies were summarized, on the basis of the leaf traits measured, in a graph (Fig. 2).

DISCUSSION

In contrast to several reports on community assemblages in harsh environments, which suggest that environmental filtering processes lead to species convergence in functional traits (Ackerly, 2004; Cornwell *et al.*, 2006; Gratani and Varone, 2006; Mouillot *et al.*, 2007), we found that the species coexisting in the coastal foredune system under study differed significantly in most of the functional traits examined. The differences in traits associated with absorption and processing of light and water conservation strongly suggest that the species under study inhabit separate functional spaces (niche differentiation). We also showed that some of the species differences in functional traits depended on time, which suggests a temporal component in the niche differentiation.

Variability in functional traits associated with light absorption

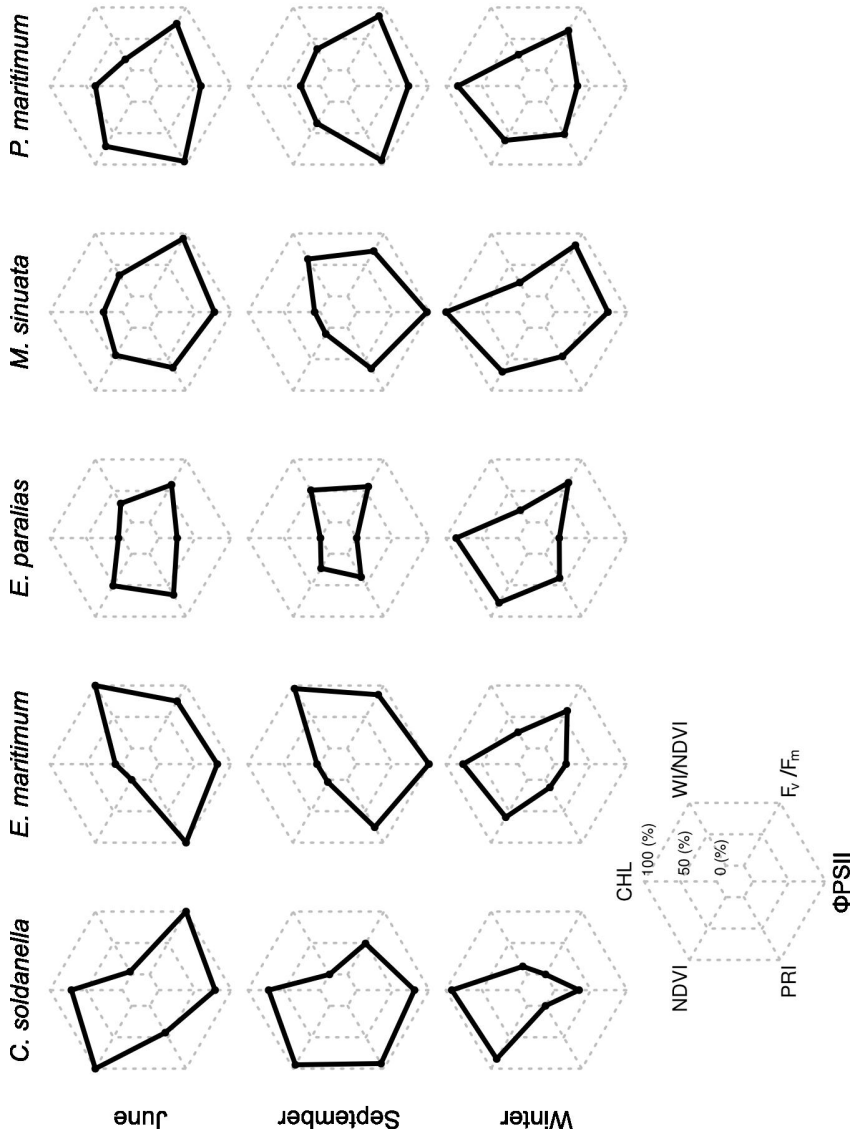


Fig. 2: A graphical summary of the different strategies of the species under study, as defined by the specific combinations of functional leaf traits. The representation of each species is based on data presented in Fig. 1, although the leaf trait values for each species are expressed in relative terms, rather than absolute terms.

The chlorophyll index (CHL) and the normalized difference vegetation index (NDVI) are both “greenness” indices that have been closely related to the chlorophyll content of leaves, as well as to the main light-harvesting or antenna pigment, the leaf nitrogen content, and to the fraction of PPFD absorbed by live vegetation (Peñuelas *et al.*, 1997; Sims and Gamon, 2002). Therefore, both parameters represent functional traits associated with the capacity of plants to capture energy, a main determinant of the productivity of plant systems (Monteith and Moss, 1977). As expected, these parameters varied less widely within the summer than between seasons, except in *C. soldanella*, in which the summer and winter values were very similar. As lower CHL and NDVI values were expected in summer, as a consequence of decreasing leaf nitrogen and chlorophyll content in response to drought (Letts *et al.*, 2010), the behaviour of *C. soldanella* is quite surprising and clearly shows that the light-harvesting capacity of this species during summer is much higher than in the other species examined.

Variability in functional traits associated with photochemical efficiency

In addition to the different capacity of plants to capture energy, it is important to determine how much of this capacity is used in practice, i.e., the efficiency with which the absorbed radiation is converted into biomass. radiation use efficiency (RUE), which is another main determinant of the net primary productivity in the widely used Monteith’s model (Monteith and Moss, 1977), varies significantly between plants, environmental conditions and ecosystems, as a result of varying environmental constraints (Field *et al.*, 1994; Garbulsky *et al.*, 2010). The effective quantum yield of photosystem II (Φ_{PSII}), which measures the proportion of the light absorbed by chlorophyll that is used in photosystem II photochemistry, has been directly related to radiation use efficiency (Genty *et al.*, 1989; Maxwell and Johnson, 2000; Roháček, 2002). In this respect, we differentiated three functional types of behaviour, represented by *E. paralias*, *M. sinuata* and the other three species.

One rather surprising result of the study was the low capacity of *E. paralias*, which has been described as a facultative C₄ and CAM plant (Daniela *et al.*, 2009), to funnel the light absorbed to the reaction centres of photosystem II. It is well known that the quantum yield of photosynthesis is higher in C₄ plants than in C₃ plants at high temperatures, and that it is lower at low temperatures because of the additional ATP required to regenerate PEP in C₄ species (Lambers *et al.*, 1998; Weng *et al.*, 2006). However, in the present study, the photochemical efficiency was lower in *E. paralias* than in the other species, in both summer and winter, with no seasonal differences in the capacity of this species to process light energy. Like *E. paralias*, *M. sinuata* displayed slight seasonal differences in its capacity to channel energy photochemically. However, unlike *E. paralias*, *M. sinuata* was active in winter, and the photochemical efficiency was higher in this species than in the other species in both winter and summer. Several studies have suggested that the functional significance of pubescence is to provide protection against light stress (Ripley *et al.*, 1999; Liakopoulos *et al.*, 2006; Skelton *et al.*, 2012), which may explain why *M. sinuata*, which has a dense pubescence, was capable of maintaining high photochemical efficiencies throughout both seasons.

Calculation of the PRI enables assessment of carbon fluxes in leaves by complementing the information provided by NDVI-like indices on the fraction of PPFD (Garbulsky *et al.*, 2011). Thus, this index of xanthophyll cycle pigment activity provided additional information about the effective quantum yield of photosystem II of the species under study. PRI values are indicative of a photoprotective mechanism that dissipates excess excitation energy via a thermal process that converts the light harvesting pigment violaxanthin to the de-epoxydised, energy quenching pigments antheraxanthin and zeaxanthin (Gamon *et al.*, 1992; Demmig-Adams *et al.*, 1996; Gilmore *et al.*, 1996; Barták *et al.*, 2004). The process of non-photochemical quenching of excess light energy is induced when, under stress conditions, the photosynthetic apparatus cannot use all of the absorbed light energy for photochemistry,

which leads to a reduction in photosystem II photochemical efficiency. Therefore, PRI would be expected to be correlated with radiation use efficiency ($\text{mol CO}_2 \cdot \text{mol}^{-1} \text{ photons}$) and inversely correlated with the dissipation of excess radiation energy as heat (Gamon *et al.*, 1992; Peñuelas *et al.*, 1995; Filella *et al.*, 1996; Gamon *et al.*, 1997). In fact, many studies have linked PRI and ΦPSII (see review in Garbulsky *et al.*, 2011). However, the results of the present study did not support the consistency of this relationship for the studied species in the stressful foredune environment, where both within summer and seasonal variations in the PRI values of species were very different from those observed for ΦPSII . This is consistent with previous reports of widely varying relationships between PRI and ΦPSII in different species (Peñuelas *et al.*, 1995; Filella *et al.*, 1996; Stylinski *et al.*, 2002; Guo and Trotter, 2004). Furthermore, it has been suggested that species-specific differences in the regulation of photosynthesis and photoprotective processes is one possible reason for the variability in these relationships (Guo and Trotter, 2004). More specifically, these authors suggested that species differences in the relationships between PRI and ΦPSII may be influenced by differences in the size of the carotenoid pool (and probably also by the xanthophyll cycle pigments) relative to total chlorophylls, since PRI has also been reported to be correlated with the ratio between total carotenoids and chlorophyll contents (Peñuelas *et al.*, 1995; Sims and Gamon, 2002; Stylinski *et al.*, 2002). In line with these findings, the PRI values, but not the ΦPSII values, were lowest in *C. soldanella*, in both July and March, which may be the result of a low ratio of carotenoids to chlorophylls in this species, which is consistent with the high chlorophyll contents. On the basis of the observed species variability in PRI values, it appears that the coexisting species under study differed in their requirements for photoprotection, suggesting functional differences in the way they use light energy.

The maximum quantum yield of photosystem II photochemistry (F_v / F_m) represents the maximum efficiency at which light absorbed by light-harvesting

antennae of photosystem II is converted to chemical energy (Butler, 1978; Baker *et al.*, 2004). In general, F_v/F_m summarizes plant health and vigour. F_v/F_m values are seasonally and diurnally stable, and they are therefore a robust indicator of functional damage to the plants (Adams III and Demmig-Adams, 2004). *Matthiola sinuata* was the only species in which the F_v/F_m values, in both summer and winter, remained higher than those reported in the literature for unstressed leaves of higher plants (0.80; Bjorkman and Demmig 1987). Values below this theoretical maximum indicate impairment or down-regulation of photosystem II efficiency (Adams III and Demmig-Adams, 2004). On the other hand, the F_v/F_m values varied most widely in *C. soldanella*; this species had the highest values across all species in summer, but the lowest values in winter (< 0.70). A decrease in values to below 0.8 has been considered symptomatic of stress-dependent photoinhibition (Bjorkman and Demmig, 1987; Maxwell and Johnson, 2000). This appears to indicate that *C. soldanella* is more sensitive than the other species coexisting in the foredune to the winter conditions (i.e. those likely to cause photoinhibition). One plausible explanation for this is that the low leaf water contents (as estimated by WI / NDVI values) in this species in winter may seriously constrain its photochemical capacity to process all of the excess of energy absorbed by the leaf pigment bed. At this point, it should be considered that the values of NDVI were highest and those of CHL were the second highest in *C. soldanella* across the species studied, and that both reflectance indices are closely related to the leaf chlorophyll content. The PRI values, which were lowest in this species in winter, are also consistent with this explanation. Low PRI values indicate low ratios of carotenoids to chlorophylls (Peñuelas *et al.*, 1995; Sims and Gamon, 2002; Stylinski *et al.*, 2002) and less protection from excess light would lead to photo-oxidative damage. As mentioned above, *C. soldanella* has C_4 metabolic characteristics, which is consistent with the lower F_v/F_m values in winter and the higher values in summer relative to those in the other species.

Variability in leaf water content

Plant water content has been reported to be significantly correlated with WI / NDVI (Peñuelas *et al.*, 1997). Plant water content is a crucial trait that influences a wide variety of physiological processes, including leaf expansion and photosynthetic carbon assimilation (Lambers *et al.*, 1998; Lu *et al.*, 1998). In the foredune environment, there may be severe competition for water owing to the low-water retention of sandy substrates, especially when high temperatures lead to high-evaporative demand. In such conditions, the ability of plants to maintain an adequate level of water in their tissues is crucial for survival (Mc Leod *et al.*, 1983; Ehleringer, 1984; Maun, 1985; Maun, 1994). The present study revealed that the extent of differences in WI / NDVI values for the coexisting foredune species varied significantly over time. Species did not differ in water content in winter, but differed greatly in summer, which is the most critical period for survival. Thus, *E. maritimum*, the species with the highest water content during summer, may possess an advantage over its co-occurring species, as it can maintain its capacity for carbon acquisition and growth. Surprisingly, the lowest leaf water contents were found in winter in all of the species except *C. soldanella*, in which values were lower in summer and similar to those in winter. This somewhat counterintuitive result may be explained by abrasion of wax and cuticular layers by winter sand-blast, which would reduce resistance to cuticular transpiration or by high atmospheric evaporative demand during winter due to a combination of factors such as strong radiation, high leaf temperature, low atmospheric water pressure and wind (Larcher, 1995).

This study showed that the individual traits were highly variable, but that the way that species combine these traits over time into different strategies also varied greatly. Considering the entire set of traits (Fig. 2), each species appears to occupy rather distinct functional space (niche differentiation in both space and time). The species with the most similar configuration of traits,

especially traits associated with light collection and leaf water content, were *E. paralias* and *M. sinuata*, although they clearly differed in the effective quantum yield of photosystem II. On the other hand, *E. maritimum* and *C. soldanella* appear to have the most different strategies. Unlike *C. soldanella*, *E. maritimum* exhibited a set of traits (the lowest chlorophyll content and vigour, the highest water content and high photochemical efficiencies) that are characteristic of plants adapted to high levels of radiation (Ehleringer *et al.*, 1997). The least plastic species, with the lowest seasonal variability in functional traits, was *P. maritimum*.

In conclusion, this research revealed that the species under study exhibit significant divergence in functional traits, which determine different strategies compatible with their coexistence in the foredune environment under study. This does not conform to previous observations in other harsh environments such as alpine, desert or Mediterranean ecosystems, where species tend to the convergence in their functional traits as a result of environmental filtering processes (Ackerly, 2004; Cornwell *et al.*, 2006; Gratani and Varone, 2006; Mouillot *et al.*, 2007). On the contrary, our data provide experimental support for the hypothesis that there is no single combination of trait values for a given environment, but rather that alternative functional designs of similar fitness might evolve in the same environment, as result of the complex interactions and trade-offs among multiple traits (Marks *et al.*, 2006). Although the present study did not aim to identify causal factors for the observed variability, the results suggest that factors claimed to promote divergence, such as limiting similarity and disturbance processes (Ranta *et al.*, 1981; Armbruster *et al.*, 1994; Stubbs and Wilson, 2004; Grime, 2006), may have an important role in structuring the dune community under study. The results provide support for the idea of a community in a dynamic equilibrium, where stable equilibrium may be prevented by multiple trade-offs associated with the interaction between stress factors and different types of disturbance, as well as by the unpredictability of foredune systems. The high level of variation in almost all

of the functional traits considered suggests a significant level of functional diversity that should affect ecosystem processes (Díaz and Cabido, 2001). Therefore, our results highlight the importance of preserving the species composition in threatened coastal dune systems in order to preserve distinctive functional process that may be unique to such systems.

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Chapter 3.

Soil characteristics and leaf traits provide evidence for differential perception of abiotic gradients in coexisting dune species.

SHORT TITLE: Species specific perception of dune gradients.

KEYWORDS: Environmental gradients; Coastal dunes; Leaf reflectance; Photochemical efficiency; Water use efficiency.

ABSTRACT

Coastal dune systems are highly stressful environments that are subject to a high degree of disturbance and are too hostile for many species to persist. The severity of the stressors usually decreases from the shoreline to inland locations, thus determining complex gradients. These gradients represent unique opportunities to study intraspecific responses to environmental changes and to investigate the abiotic factors that drive community change. In this study, we examined whether the species that coexist along a supposedly directional dune gradient perceive environmental changes as non-directional.

We also investigated whether the variation in species leaf traits is correlated with changes in environmental factors along the gradient. Finally, we studied how the degree of convergence in leaf traits differs at both extremes of the dune gradient. With these aims, we measured a series of leaf traits (related to plant efficiency in use of light, water and nutrients) in two species coexisting along a 600 m long dune gradient. We also measured possible stressors (salt content and pH) and potentially important limiting resources (water and nutrient contents) in the soil under the plants. Variations in most of the soil variables, except nitrogen content, were spatially dependent. The variations were only directional for soil water content and salinity, the values of which decreased from coastal to inland positions. Species differed along the dune gradient in soil nutrient contents and pH. Although the patterns of variation in structural traits were spatially dependent, the level of dependence differed for almost all possible combinations of species and traits. Variations in functional traits also depended on the position on the gradient, except for the effective quantum yield of photosystem II and $^{15}\text{N} / ^{14}\text{N}$ isotopic ratio. The pattern of variation was directional for reflectance indices related to light absorption and use. Different species were found to perceive and respond to variations along gradients in different ways, thus highlighting the overly simplistic nature of previous attempts to determine the main environmental factors explaining zonation along dune gradients.

INTRODUCTION

Coastal sand dune systems occur at the interface between marine and terrestrial environments. These contrasting influences create challenging conditions for plant growth (Wartena *et al.*, 1991; Griffiths, 2006; Ihm *et al.*, 2007). The species that inhabit such habitats must endure the harsh conditions caused by multiple stress factors and disturbances at different temporal and spatial scales (Franks, 2003). According to Grime (1977), these highly

stressful disturbance-prone environments may be too hostile for many species to persist. The intensity of these factors often decreases gradually from the shoreline to inland locations, thus determining complex environmental gradients (Ishikawa *et al.*, 1995; Costa *et al.*, 1996; Franks, 2003; Sykora *et al.*, 2004; Dech *et al.*, 2005; Griffiths, 2006). The most hostile environmental conditions occur at the coastal end of such gradients because of the severity of abiotic factors (e.g. high airborne and soil salinity, low availability of nutrients, sand burial and blasting, etc.) (Ishikawa *et al.*, 1995; Houle, 1996; Costa *et al.*, 1996; Houle, 1997; Griffiths, 2006; Lortie and Cushman, 2007; Kim and Yu, 2009), although the intensity of abiotic factors is known to vary widely at relative short distances from the foredune (Olf *et al.*, 1993; Houle, 1996; Lortie and Cushman, 2007). Coastal dune species have evolved numerous adaptations in response to these varying conditions: pubescence and epicuticular waxes (Ehleringer *et al.*, 1976; Maun, 2009); folding and rolling of leaves to diminish water loss due to high radiation and temperatures (Maun, 1994; Maun, 2009); succulence and storage organs to control the water supply (de Jong, 1979; Maun, 1998; Migahid, 2003; Suárez, 2011); accumulation of solutes (Rozema *et al.*, 1985; Khedr *et al.*, 2003); development of C₄ and CAM metabolism (Elhaak *et al.*, 1997; Daniela *et al.*, 2009); special germination strategies (Maun, 1994); plasticity in functional traits (Maun, 1998; Davy *et al.*, 2006); establishment of mycorrhizal interactions (Maun, 1994; Cakan and Karatas, 2006); differences in morphology (Voronkova *et al.*, 2008; Daniela *et al.*, 2009) and life-forms (García-Mora *et al.*, 1999; Cakan and Karatas, 2006), etc. (see Rozema *et al.* (1985), Hesp (1991) and Maun (2009) for an extensive review).

Many studies on community assembly have suggested that species growing under harsh abiotic conditions, such as those existing at the coastal end of dune gradients, would tend to converge in their functional traits because of design restrictions imposed by the environment, in a process known as environmental filtering (Houle, 1996; Webb *et al.*, 2002; Cornwell

et al., 2006; Grime, 2006; Mouillot *et al.*, 2007). However, in environments in which the main stress factors are related to competitive processes rather than to physical factors, as may occur at the inland end of the dune gradient, limiting similarity has been proposed as an important process promoting divergence in functional traits and species niche differentiation (MacArthur and Levins, 1967; Abrams, 1983; Stubbs and Wilson, 2004; Mouillot *et al.*, 2007; Ashton *et al.*, 2010; Maltez-Mouro *et al.*, 2010; Thorpe *et al.*, 2011). Therefore, species inhabiting the foredune would be expected to develop very different strategies from those that inland species develop. The former would be restricted to the foredune area as a result of their lower competitive capacity, whereas the more inland species could not thrive at the foredune end because of their lower tolerance to the harsh abiotic conditions. As spatial variations in many environmental factors occur gradually along the dune gradient, species become distributed in zones, according to how they tolerate the different conditions (Daubenmire, 1968). However, exceptionally, a few dune species are able to occupy the entire dune gradient. For example, the perennial herbs *Eryngium maritimum* and *Pancreatium maritimum* are found growing from shoreline to inland locations.

Dune gradients represent unique opportunities to study intraspecific responses to environmental changes in natural conditions and to investigate the abiotic factors that may drive community change (Goldberg, 1990; Bertness *et al.*, 1994; Callaway, 1997; Lortie and Cushman, 2007). Moreover, coastal dunes are worthy of study because they represent vulnerable systems threatened by factors such as urban expansion, species invasion and the rise in sea level as a result of climate change. These on-going changes may remove processes that are unique to these systems and that have important effects on ecosystem functioning and the services that they provide.

In this study, we addressed three main questions. Firstly, we considered whether *E. maritimum* and *P. maritimum* perceive variations in edaphic variables along the dune gradient as directional. Previous studies have

reported that many severe stressors, such as salt spray, sand burial, swash inundation, dryness, high light intensity, wind exposure and nutrient deficiency, increase or decrease consistently with increasing distance from the shoreline (Kachi and Hirose, 1979; Maruyama and Miura, 1981; Miura and Maruyama, 1983; Hesp, 1991). However, if the gradient is long enough, individuals located along the gradient could perceive supposedly directional changes in abiotic factors as non-directional. We predicted that the following could disrupt the perception of environmental gradients by plant species: microhabitat selection or habitat modifications by species, spatial heterogeneity caused by landform and geomorphic processes, and complex interactions between factors. Secondly, we considered whether *E. maritimum* and *P. maritimum*, which coexist along the entire dune gradient, differ in their response strategies to the environmental changes that occur along the gradient. As both species widely differ in leaf morphology, phenology and life form, we expected to find a significant level of interspecific variation in their ecophysiological responses. To address this issue, we attempted to determine whether variation in structural and functional leaf traits of *E. maritimum* and *P. maritimum* were in some way correlated with changes in individual environmental factors that occur along the coast-land gradient. Since previous studies have reported that plant zonal distribution patterns are correlated with variables such as soil salinity (Kachi and Hirose, 1979; Maruyama and Miura, 1981; Miura and Maruyama, 1983), soil-water content (Kachi and Hirose, 1979; Maruyama and Miura, 1981), salt spray (Boyce, 1954; Barbour, 1978), and sand movement (Nobuhara, 1967), we expected to find some consistent relationships between variation in structural and functional leaf traits, which influence the fitness of the species, and in edaphic variables, which presumably show consistent gradients from the shoreline to the inland. Finally, we considered whether there is greater convergence in the leaf traits of the species inhabiting the shoreline end of the dune gradient, where harsher environmental conditions are expected, than in those inhabiting the inland

extreme of the gradient. In line with previous studies on community assemblages (Paruelo *et al.*, 1998; Reich *et al.*, 2003; Meinzer, 2003; Bucci *et al.*, 2004; Mouillot *et al.*, 2007; Swenson and Enquist, 2007), we expected higher convergence in traits at the shoreline end due to environmental filtering processes.

To achieve the above-mentioned aims, we measured a series of leaf traits related to plant efficiency in the use of essential resources, such as light, water and nitrogen, in two species that coexisted along a 600 m long dune gradient. Variations in physicochemical soil variables may be important in explaining leaf variation in physiology and in determining the spatial patterns of the species. Therefore, we also measured several parameters in the soil in which the plants were growing, as these parameters may act as stressors (salt content and pH) or may be important limiting resources (water and nutrients).

MATERIALS AND METHODS

Species and location

Two perennial herbaceous species coexisting in the coastal dune system of Valdoviño (43°36'44"N, 8°10'58"W; Galicia, NW of Spain) were selected for study. The species were distributed along a 600 m long gradient from the shore to the terrestrial environment (hereafter shore-to-land gradient). The slope in the dune system is 4.2 %, and the orientation is NNE. *Pancratium maritimum* L. (Amaryllidaceae) is a cryptophyte with a bulbous stem and leaves that emerge from a basal rosette. The glaucous blue-green leaves are thick, long and narrow. The plant has 3-15 hermaphroditic, funnel-shaped white flowers that appear late summer (August - October) in an umbel at the top of reproductive stems. *Eryngium maritimum* L. (Umbelliferae) is an erect hemicryptophyte perennial herb, with a long deep root system, which can reach to a depth of 50 cm in sand or shingle. The blue-green leaves are three-

lobed, folded, stiff and waxy with a leathery texture and prickly borders.

Sampling design

In September 2008, four 20×8 m plots were established along a shore-to-land gradient running perpendicular to the coastline. The plots were sited with the longest side parallel to the coast, at distances of 0, 200, 400 and 600 m from the foredune. Soil samples were collected in each plot, along with randomly selected mature plants of *P. maritimum* and *E. maritimum*, which were used to measure instantaneous and time integrated functional leaf traits, as described below.

Leaf spectral reflectance

The spectral reflectance of fully expanded leaves was measured in twelve randomly selected mature individuals per species and plot. The reflectance spectra, within a wavelength ranging from 300 to 1100 nm, were recorded with a portable spectroradiometer (Unispec, PP Systems Haverhill, MA, USA). Leaf reflectance was calculated by dividing the spectral radiance of the leaf by the radiance of a reflective white standard (Spectralon Reflectance Standard, Labsphere, North Sutton, NH, USA). Reflectance data thus obtained were processed to calculate the following indices related to functional traits and physiological processes: the chlorophyll index (CHL), measured as the R_{750} / R_{550} ratio, where R_{750} and R_{550} represent the reflectance at 750 and 550 nm, respectively. The R_{750} wavelength is relatively insensitive to chlorophyll content changes, whereas R_{550} is sensitive to variations in chlorophyll content of leaves. This index is highly correlated with leaf chlorophyll content (Wood *et al.*, 1993; Lichtenthaler *et al.*, 1996; Gitelson *et al.*, 1997). The photochemical reflectance index (PRI), calculated as $(R_{531} - R_{570}) / (R_{570} + R_{531})$ (Gamon *et al.*, 1992; Peñuelas *et al.*, 1995), is a normalized index (ranging from -1 to 1) related to the violaxanthin-zeaxanthin xanthophyll cycle. This is

a light dependent reaction associated with thermal dissipation of energy that decreases the photochemical efficiency of photosystem II (Gamon *et al.*, 1992; Peñuelas *et al.*, 1995; Filella *et al.*, 1996). The normalized difference vegetation index (NDVI), computed as $(R_{800} - R_{680}) / (R_{800} + R_{680})$, is a normalized vigour index (Peñuelas *et al.*, 1994; Gamon *et al.*, 1995). The wavelength in the visible range (680 nm) is sensitive to chlorophyll content, whereas that in the near infrared range (800 nm) is sensitive to variations in water content. NDVI has been correlated with leaf chlorophyll content, photosynthetic efficiency, foliar nitrogen, phosphorus and potassium content (Peñuelas *et al.*, 1993; Gitelson *et al.*, 1994; Gamon *et al.*, 1995; Albayrak *et al.*, 2011).

Chlorophyll fluorescence indices

The effective quantum yield of photosystem II (Φ_{PSII}) was measured close to the centre of the leaves (the same leaves used to measure reflectance spectra), but avoiding the main nerves. This parameter is related to the dissipation of the excess energy obtained from photons as fluorescence. It was calculated from data obtained with a portable pulse-amplitude-modulated fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany) as $\Phi_{PSII} = (F'_m - F_t) / F'_m$ (Maxwell and Johnson, 2000; Roháček, 2002), where F_t is the minimal fluorescence emission under an illuminated environment and F'_m is the maximal intensity of fluorescence emitted after a light saturating pulse, also under light conditions. Data were obtained under incident photosynthetic photon flux density (PPFD) of $900 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \pm 21 \text{ S.E.}$, with an external halogen lamp attached to the fluorometer. Measuring light and saturating light pulses ($> 4000 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 0.8 s pulse length, actinic white light) were applied through a fibre-optic probe at an angle of 60° relative to the sample and 12 mm from the leaf, with an Arabidopsis leaf clip holder 2060-B (Heinz Walz GmbH, Effeltrich, Germany).

Relative water content

The relative water content (RWC) of leaves indicates the proportion of water accumulated in leaves in relation to saturation. The RWC was estimated in sample leaves from eight individuals per species and plot. The leaves were transported to the laboratory in hermetically sealed, ziplock plastic bags. The RWC was calculated as $[(M_f - M_d) / (M_t - M_d)] \cdot 100$ (Peñuelas *et al.*, 2004; Chen *et al.*, 2009), where M_f is the fresh mass of leaves, M_t is the turgid mass, measured after saturation of leaves in filter paper soaked with distilled water in covered Petri dishes for 20 h at 4°C in the dark (leaves were blotted dry with tissue paper before determination of M_t), and M_d is the dry mass of leaves after drying in an oven at 80°C to constant weight.

Specific leaf area

Specific leaf area (SLA), i.e. the leaf area per unit dry mass, measures the light-intercepting leaf area per unit of leaf-dry-mass investment. Species with low SLA have a thicker leaf blade or denser tissue, or both (Wright *et al.*, 2004). The projected area of one leaf per individual was captured in eight individuals per species and plot, with a flatbed scanner (CanonScan LiDE 50, Canon Inc., Tokyo, Japan) and processed with image analysis software (ImageJ 1.431, National Institute of Health, USA; Rasband, 1997-2011). Samples were then dried in an oven at 80°C to constant weight and weighed to determine the dry mass. The SLA was calculated as the quotient between the area and the dry mass ($\text{mm}^2 \cdot \text{g}^{-1}$).

Stomatal density

The stomatal density (SD) of nine individuals per species and plot was determined in fully expanded leaves as the number of stomata of abaxial and adaxial sides of leaves per leaf area. Leaf imprints were obtained by applying a thin layer of clear nail varnish to both sides of leaves. The impressions were

peeled off and mounted on slides. Then $100 \times$ micrographs were captured and the stomata were counted in three frames of $500 \times 500 \mu\text{m}$ per slide. Stomatal density was expressed as number of stomata per mm^2 .

Leaf carbon and nitrogen content and isotopic composition

Between two and five pieces of mature leaves from six randomly selected plants per species and plot were cleaned, to remove organic debris, and dried at 60°C for more than 5 days, before being ball-milled and sieved. Subsamples of 1-2 mg of homogenized fine powder of particle size $< 500 \mu\text{m}$ diameter were encapsulated in tin capsules. The molar $^{15}\text{N} / ^{14}\text{N}$ and $^{13}\text{C} / ^{12}\text{C}$ ratios were determined in an Isotope Ratio Mass Spectrometer (ThermoFinnigan MAT253, Bremen, Germany). The foliar carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope compositions were expressed relative to the composition of a standard (Pee Dee Belemnite calcium carbonate for C, and air for N). The δ values (‰) were calculated as $\delta = [(R_{\text{sam}} / R_{\text{std}}) - 1] \cdot 1000$, where R refers to the $^{13}\text{C} / ^{12}\text{C}$ or $^{15}\text{N} / ^{14}\text{N}$ ratio in the plant sample (R_{sam}) and the standard (R_{std}), respectively. For analytical control purposes, polyethylene (IAEA-C6) and $(\text{NH}_4)_2\text{SO}_4$ (IAEA N1) were used as secondary international isotope standards for C and N, respectively. The $\delta^{13}\text{C}$ values were transformed into carbon isotope discrimination ($\Delta^{13}\text{C}$) values by use of the expression: $\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}}) / (1 + \delta^{13}\text{C}_{\text{plant}})$; Farquhar *et al.*, 1989), assuming a $\delta^{13}\text{C}$ air value of - 8.0 ‰ on the PDB scale. Carbon and nitrogen contents were determined (and expressed as percentage of dry mass), in the same samples, in an elemental analyzer (FlashEA 1112 Series; ThermoFinnigan, Bremen, Germany) coupled to the mass spectrometer. The analyses were performed in the Instrumental Analysis Unit at the University of A Coruña (Spain). In C_3 plants, the $\Delta^{13}\text{C}$ values generally lie between ~ 15 ‰ (for plants with higher water use efficiencies; WUE) and 25 ‰ (for plants with lower efficiencies; Dawson *et al.*, 2002).

Level of convergence in leaf traits

The absolute differences in mean values of species for each trait at both ends of the dune gradient were compared to determine whether convergence in leaf traits of species differed at these extremes.

Physicochemical soil variables

One soil sample was obtained at 10 cm from the base of each of nine plants per species selected for ecophysiological measurements in each plot. Samples were collected with a cylindrical steel sampler of diameter 5 cm and length 25 cm. The samples were transported to the laboratory in airtight plastic containers of volume 120 ml. The samples were weighed immediately upon arrival to the laboratory (M_f) and dried at constant weight at 80°C (M_d), and the soil water content was estimated as follows: $[(M_f - M_d) / M_d] \cdot 100$ (g water · Kg⁻¹ dry soil).

Soil salinity was determined by electrical conductivity in a 1 : 2.5 soil: water suspension, with an EC meter (524 Crison, Crison Instruments, Barcelona, Spain). Soil pH was determined in a similar soil : water suspension, with a pH meter (pH-metro 507, Crison Instruments, Barcelona, Spain). Different soil subsamples were used to quantify the amount of inorganic phosphorus available for plants, by the Olsen method (Olsen *et al.*, 1954). The proportions of total soil carbon (C_{tot}) and nitrogen (N_{tot}) were determined in finely ground (< 2 mm) subsamples of soil (~ 2 mg). Subsamples were encapsulated in tin capsules and analyzed (in the Department of Edaphology of the University of Santiago de Compostela) by combustion at high temperature in a CHNS elemental analyzer (LECO CHNS-932; LECO Corp., St. Joseph, MI, USA).

Statistical analysis

The effects of Species and Position on functional leaf traits and soil variables was analyzed by two-way Analysis of Variance (ANOVA). The

“aov” function included in “stats” package of the R statistical software environment (R Development Core Team, 2009) and “Anova” function included in “car” package (Fox, 2009) were applied. The significance threshold (α) was set to 0.05. Prior to analyses, all variables were checked for the normality and homoscedasticity required by the Analysis of Variance. No variable severely deviated from the underlying assumptions, thus justifying the use of ANOVA (Zar, 1984).

RESULTS

Leaf reflectance and Chlorophyll fluorescence

Chlorophyll content, as estimated by CHL values, differed significantly between species, and the values were highest in *P. maritimum* at all sampling points along the gradient. Chlorophyll content also varied significantly along the gradient, with an overall decrease from the coastal to the inland plots, especially in *P. maritimum* (Table 1; Fig. 1a).

The species differed in the patterns of variation in the vigour index (NDVI) along the shore-to-land gradient. NDVI values for *E. maritimum* remained fairly constant along the gradient, whereas NDVI values in *P. maritimum* increased steadily from coastal to inland positions (Table 1; Fig. 1b).

The PRI values increased significantly along the gradient, from coastal to inland plots. The values were higher in the former than in the latter along the whole gradient (Table 1; Fig. 1c). However, the effective quantum yield of photosystem II (Φ PSII) showed no significant differences between species or among positions along the gradient (Table 1; Fig. 1d).

Structural traits

Analyses of direct estimations of leaf water content by RWC did not reveal any significant differences between species. The RWC values varied significantly along the gradient, but with no consistent trend (Table 2; Fig. 2a).

The SLA was consistently higher (i.e. denser leaves) in *E. maritimum* than in *P. maritimum* along the entire gradient. Both species exhibited very similar trends of variation in SLA values, which increased from the coastal to inland sites, reaching the maximum values at a distance of 400 m from the foredune (Table 2; Fig. 2b).

The species showed contrasting patterns of variation in stomatal density (SD). Thus, the SD values increased steadily in *P. maritimum* from coastal to inland sites, and they followed the opposite trend in *E. maritimum*. The SD values were higher in *E. maritimum* than in *P. maritimum* at all positions along the gradient (Table 2; Fig. 2c).

Nitrogen and carbon leaf content

The species differed in the proportion of nitrogen in their leaves, and these differences varied significantly at different positions along the gradient. In particular, leaf nitrogen content in *P. maritimum* increased steadily from the coastal sites, reaching the highest values at 400 m, after which it abruptly decreased to the minimum value at the inland end of the gradient. The opposite pattern of variation in leaf nitrogen contents was observed in *E. maritimum* and the values were almost always much lower than in *P. maritimum*, except at the inland end of the gradient, where they reached much higher levels than in *P. maritimum* (Table 3; Fig. 3a). The variation in leaf carbon content followed exactly the same pattern described for leaf nitrogen in both species (Table 3; Fig. 3c).

Nitrogen and carbon isotopic discrimination

Leaf $\delta^{15}\text{N}$ did not differ significantly between species or along the gradient

(Table 3; Fig. 3b). Marginally significant differences in the water use efficiency in species, as estimated by $\Delta^{13}\text{C}$, depended on positions of the

Table 1 Results of factorial analysis of variance for leaf functional traits chlorophyll content (CHL), vigour (NDVI) and photochemical efficiencies (PRI and ΦPSII) for the factors Position and Species. Significant differences ($P < 0.05$) are shown in bold type

df	CHL			NDVI			PRI			ΦPSII			
	SS	F	P	SS	F	P	SS	F	P	SS	F	P	
Position	3	0.76	9.96	<0.001	39	29468	<0.001	0.0052	10.76	<0.001	0.031	1.67	0.181
Species	1	5.67	221.98	<0.001	5	11720	<0.001	0.0085	52.69	<0.001	0.000	$3.0 \cdot 10^{-4}$	0.986
Position \times Species	3	0.19	2.49	0.067	23	17690	<0.001	$8.9 \cdot 10^{-4}$	1.85	0.145	$4.2 \cdot 10^{-3}$	0.23	0.877
Residuals	76	1.94			33			0.0122			0.471		

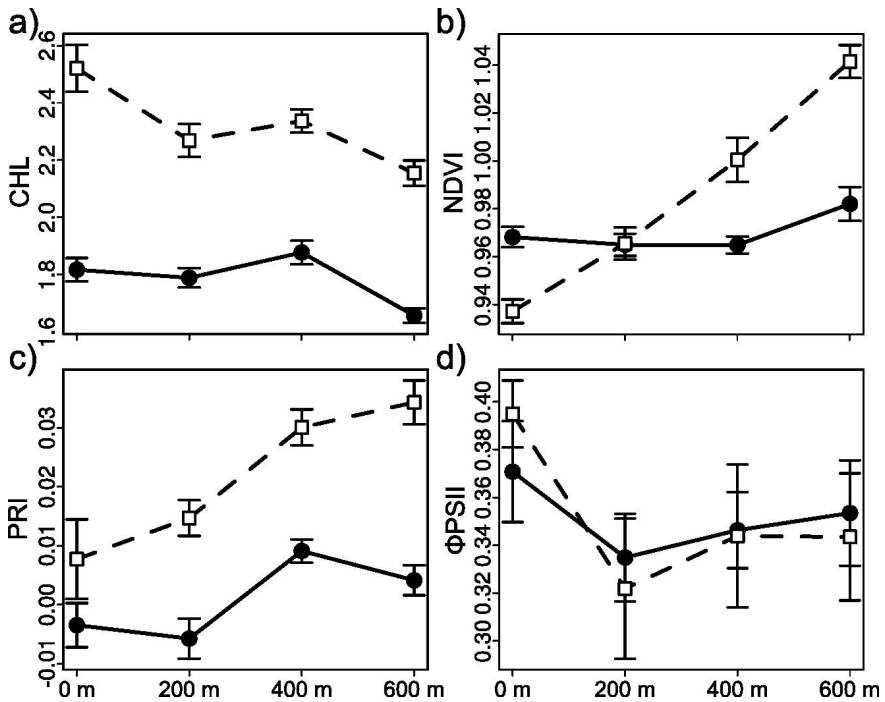


Fig. 1. Values of leaf functional traits based on leaf reflectance and chlorophyll fluorescence measurements at different positions along the dune gradient: a) chlorophyll index b) normalized differential vegetation index, c) photochemical reflectance index, and d) effective quantum yield of photosystem II. Open squares represent mean values (\pm S.E., $n = 12$) for *P. maritimum* and solid circles represent the mean values for *E. maritimum*.

species along the gradient. The WUE was lowest (highest $\Delta^{13}\text{C}$) in *E. maritimum* at both ends of the gradient (Table 3; Fig. 3d).

Level of convergence in leaf traits

Examination of the extent of species differences in leaf traits at both ends of the gradient revealed greater convergence in five out of eleven leaf traits examined (PRI, NDVI, SLA, and N and C leaf content) at the shoreline end

Table 2. Results of factorial Analysis of Variance for relative water content (RWC), specific leaf area (SLA) and stomatal density (SD). Significant differences ($P < 0.05$) are shown in bold type.

	df	RWC			SLA			SD			
		SS	F	P	SS	F	P	SS	F	P	
Position	3	447	21.21	<0.001	2616	4.35	0.008	3	792	0.20	0.895
Species	1	19	2.72	0.105	4810	23.97	<0.001	1	97780	74.80	<0.001
Position × Species	3	30	1.43	0.242	367	0.61	0.611	3	11582	2.95	0.039
Residuals	56	394			11237			64	83665		

than at the inland end of the dune gradient.

Soil physicochemical variables

The species did not differ in the soil water content, which decreased

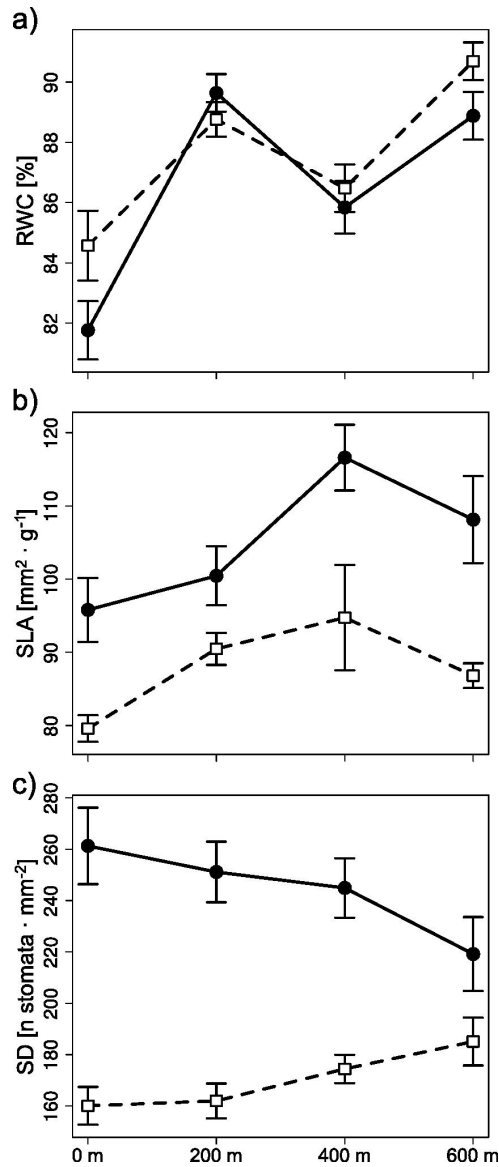


Fig. 2. Values of structural leaf traits of the species studied at different positions along the dune gradient: a) relative water content (RWC), b) specific leaf area (SLA), and c) stomatal density (SD). Open squares represent mean values (\pm S.E., $n = 8-9$) for *P. maritimum* and solid circles represent the mean values for *E. maritimum*.

significantly for both species from coast to inland positions (Table 4; Fig. 4a). Soil salinity varied for both species and followed a very similar pattern to that described for soil water content (Table 4; Fig. 4b).

The soil pH along the gradient differed significantly in relation to the plant species, although no clear trend in the variations was observed. However, at the inland end of the gradient, the soil pH was similar for both species (Table 4; Fig. 4c). Species did not differ significantly in soil carbon content. For both species, the carbon content of the soil was higher at the ends of the gradient than in the middle positions, and reached lowest values at a distance of 400 m from foredune (Table 4; Fig. 4d). The soil carbon content was mainly derived

Table 3. Results of factorial Analysis of Variance for nitrogen and carbon content in leaves and stable isotope discrimination. Significant differences ($P < 0.05$) are shown in bold type (for carbon discrimination are marked the marginally significant differences).

df	N _{plant}			δ ¹⁵ N			C _{plant}			Δ ¹³ C			
	SS	F	P	SS	F	P	SS	F	P	SS	F	P	
Position	3	0.29	0.18	0.909	291	0.20	0.653	5	0.33	0.802	2	3.83	0.055
Species	1	2.29	4.23	0.044	877	0.21	0.892	12	2.11	0.151	3	1.59	0.201
Position × Species	3	18.47	11.38	<0.001	9336	2.19	0.099	68	4.13	0.009	5	2.65	0.057
Residuals	63	34.09			89714			345			37		

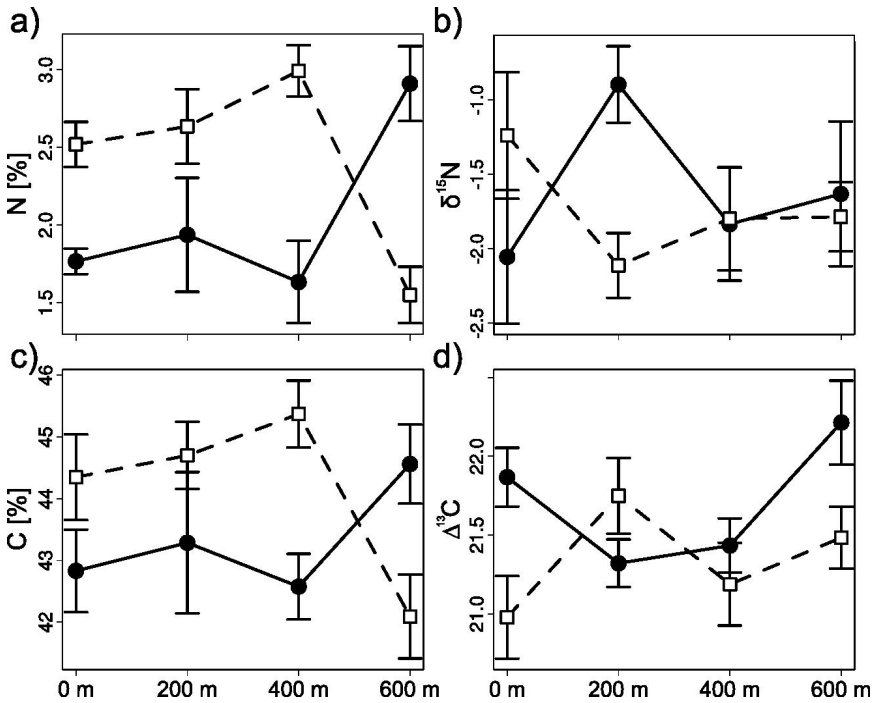


Fig. 3. Values of leaf carbon and nitrogen content, carbon isotopic discrimination ($\Delta^{13}\text{C}$) and nitrogen isotope compositions ($\delta^{15}\text{N}$) at different positions along the dune gradient: a) leaf nitrogen content, b) leaf $^{15}\text{N} / ^{14}\text{N}$ ratio, c) leaf carbon content, d) leaf $^{13}\text{C} / ^{12}\text{C}$ ratio. Open squares represent mean values (\pm S.E., $n = 8-10$) for *P. maritimum* and solid circles represent the mean values for *E. maritimum*.

from carbonates (C_{tot} was composed principally of carbonates plus 0.1 to 0.2% from other sources). The soil nitrogen content did not differ significantly along the gradient. It was consistently higher along the whole gradient for *E. maritimum* than for *P. maritimum* (Table 4; Fig. 4e). Differences in soil phosphorus depended significantly on the position of the plant species along the gradient. The soil phosphorus content did not differ in relation to plant species at the two positions closest to the coastal end of the gradient. The phosphorus content in *E. maritimum* soils remained almost constant from the coastal end of the gradient towards inland positions, whereas in *P. maritimum*

it increased steeply and reached maximum values at the inland end of the gradient (Table 4; Fig. 4f).

DISCUSSION

On the differential perception of abiotic gradients by dune species

The results of the present research provide evidence that the species under study both perceived differently the variations in some abiotic factors that occur along a dune gradient. Environmental gradients in dune systems have classically been described as directional, with consistent changes from shoreline to inland positions. Thus, it has been reported that soil water content increases and soil salinity decreases with increasing distance from the shoreline (Kachi and Hirose, 1979; Maruyama and Miura, 1981; Miura and Maruyama, 1983). Other severe stressors in the form of salt spray, sand burial, swash inundation, dryness, high light intensity, wind exposure and nutrient deficiency have been reported to predominate in the foredune environment and to decrease with distance from the coast (Hesp, 1991). However, the results of the present study indicate that some of the putative abiotic gradients investigated are not perceived as such by the species studied (i.e. values in these abiotic factors are not significantly affected by position in the gradient) or are perceived in a more complex way as non-directional (the sequence of values do not increase or decrease steadily with distance from the shoreline). Thus, in contrast to previous studies reporting a correlation between soil nutrient content and distance inland from the beach (Hesp, 1991; Lortie and Cushman, 2007), we did not find such relationship for soil nitrogen content along the dune gradient; we also found that nitrogen content was always lower in soils under *P. maritimum* than in soils under *E. maritimum*. The patterns of variation for the other physicochemical characteristics of the soils of the species studied were spatially dependent and sometimes species specific.

However, except for salinity of the soils under *E. maritimum*, there was no consistent directional change in any case. Thus, the present results do not

Table 4. Results of factorial Analysis of Variance for the soil variables water content of soil, salinity, pH, total amount of carbon, total amount of nitrogen and available phosphorus for the Species and Position factors. Significant differences ($P < 0.05$) are shown in bold type.

df	%H			Salinity			pH			C			N			P			
	SS	F	P	SS	F	P	SS	F	P	SS	F	P	SS	F	P	SS	F	P	
Position	3	16.07	10.46	<0.001	32.74	5.02	0.003	0.2	6.42	0.001	1051	3.22	0.033	0.0010	1.80	0.162	1572	3.96	0.015
Species	1	0.29	0.56	0.456	0.38	0.17	0.679	0.0	$1.8 \cdot 10^{-3}$	0.966	19	0.17	0.678	0.0075	39.61	<0.001	1417	10.71	0.002
Position \times Species	3	1.29	0.84	0.475	11.68	1.79	0.157	0.5	12.74	<0.001	291	0.89	0.455	0.0004	0.73	0.542	3013	7.59	<0.001
Residuals	40	34.82			147.91		0.5				4360			0.0076			5291		

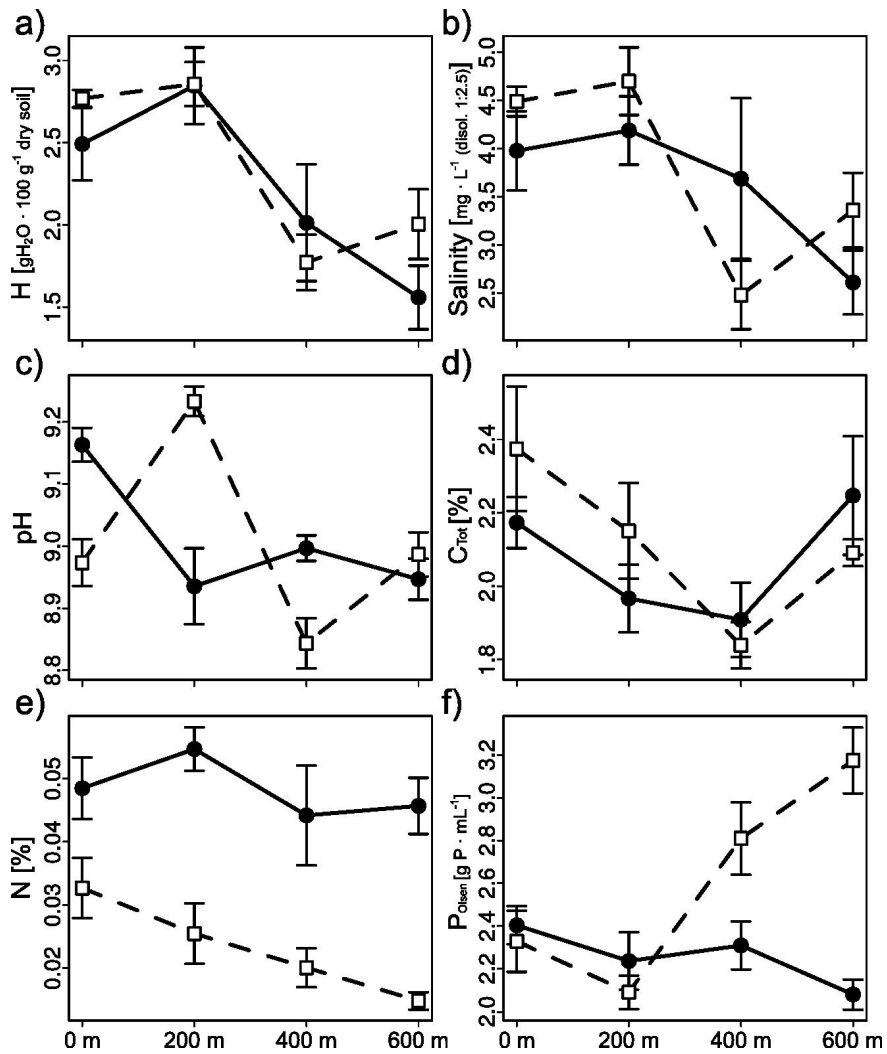


Fig. 4. Values of soil physicochemical variables along the dune gradient: a) soil water content, b) soil salinity, c) pH, d) total carbon content, e) total nitrogen content, and f) available phosphorus. Open squares represent mean values (\pm S.E., $n = 6$) for soils under *P. maritimum* and solid circles represent mean values for soils under *E. maritimum*.

conform to the general observation that most abiotic gradients in dune systems are directional (Kachi and Hirose, 1979; Maruyama and Miura, 1981; Miura and Maruyama, 1983; Hesp, 1991). In a study of abiotic gradients in much shorter coastal dunes (200 m long) than sampled in the present study, Lortie and Cushman (2007) reported the presence of both directional (soil nitrate) and non-directional (ammonium, pH) gradients. One plausible explanation for the difference in the results is that, in the present study, the soil samples were obtained close to the plant roots and were therefore influenced by root activity. Hence, our results suggest that plant species may select micro habitats originated by landform and geomorphic processes (Kim and Yu, 2009) or they may modify the soil environment in a specific way. The patterns of spatial variation in soil phosphorus content and soil pH were different for each species. We recognise that complex interactions of factors may also affect the directionality of the changes in edaphic variables along dune gradients. For example, the variation in soil phosphorus was spatially dependent for *P. maritimum*, but not for *E. maritimum*. These differences could be explained by differences in the tendency of species to establish mycorrhizal associations. At the elevated pH values existing in the dune system, phosphorus can be immobilized as insoluble carbonates (Faurie *et al.*, 1990; Kooijman *et al.*, 1998; Kooijman *et al.*, 2009; Garg *et al.*, 2010). The increased available phosphorus in soils under *P. maritimum* in the more inland sites could be explained by the ability of this species to establish specific mycorrhizal associations, which are common in this type of environment (Camprubi *et al.*, 2011). The low P availability at the two sites closest to the coast could be explained by inhibition of fungal colonization probably caused by environmental stress factors such as soil salinity (Mohammad *et al.*, 2003; Cakan and Karatas, 2006; Camprubi *et al.*, 2010).

Species differences in response strategies to abiotic changes in dune gradients

Structural traits

The patterns of variation along the dune gradient in the structural traits examined revealed significant spatial dependence for all traits, but it was clearly different for almost all possible combinations of species and traits. The pattern of variation was only directional for stomatal density, although with opposite trends in each species.

SLA has been considered the main determinant of growth variation, at least for herbaceous plants (Lambers *et al.*, 1992; Poorter *et al.*, 1998). In the species under study here, SLA increased with a similar pattern from the coastal sites, where the abiotic conditions were most stressful, towards inland sites, reaching maximum values at 400 m from the shoreline. A low SLA, i.e. a large amount of photosynthetically active material per unit leaf area, may be beneficial for species at the high levels of radiation experienced in dune habitats, thus reducing the risk of photoinhibition (Sánchez-Vilas *et al.*, 2011). Low SLA may be particularly advantageous at the shoreline, since low values has been considered beneficial for water conservation and for tolerating the high winds characteristics of these sites (Givnish, 1979; Retuerto *et al.*, 1992; Retuerto and Woodward, 1993). This suggests that *P. maritimum*, in which SLA values were consistently lower than in *E. maritimum*, may be better adapted to the conditions at the shoreline end of the dune gradient. However, the higher stomatal density, a xeromorphic character, suggests that *E. maritimum* should be less vulnerable to drought than *P. maritimum*. The higher stomatal density in *E. maritimum* than in *P. maritimum* may enhance adaptation to drought via more efficient control of water loss by changes in stomatal conductance. In fact, stomatal density has been positively related to stomatal conductance, net CO₂ assimilation rate and water use efficiency (Spence *et al.*, 1986; Retuerto and Woodward, 1993; Xu *et al.*, 2008). The opposite trend in stomatal density, which decreased in *E. maritimum* and increased in *P. maritimum* from shoreline to inland sites, suggests contrasting strategies in these species involving diffusion of CO₂ into the leaf and the

outward diffusion of water vapour (Hetherington *et al.*, 2003). The WUE was also higher in *E. maritimum* at both ends of the gradient, as indicated by the $\Delta^{13}\text{C}$ values; this supports the previously reported positive correlations between stomatal density and $\Delta^{13}\text{C}$, both of which are closely linked to carbon fixation and water status in C_3 plants (van de Water *et al.*, 1994).

Under the conditions at both ends of the dune gradient, where water may be less available, the RWC was higher in *P. maritimum* leaves than in *E. maritimum* leaves, which is consistent with the lower WUE and SLA. The higher RWC in *P. maritimum* may be caused by a higher capacity to accumulate proline (Khedr *et al.*, 2003), an osmotically active amino acid that contributes to water retention and to the protection of proteins and membranes from the detrimental effects of high salinity (Larcher, 1995). However, the mean RWC values were higher than 80% in both species along the entire gradient. As this is much higher than the values at which wilting may appear (Ludlow, 1989), both species would presumably be able to maintain their leaf water potential for carbon acquisition and growth.

The lack of correspondence between the patterns of variation in structural traits and in the soil physicochemical parameters indicates that none of the physicochemical parameters appears to explain the spatial variation of the structural traits for any of the species. Each combination of trait and species appears to respond in a different way to a complex environmental gradient or to a single environmental factor that was not considered in this study. Under these conditions, it is very difficult to interpret the observed patterns of variation in terms of adaptation.

Functional traits

The greenness indices CHL and NDVI have been related to the chlorophyll content of leaves (Peñuelas *et al.*, 1997; Sims *et al.*, 2006), which is linked to the capacity of live vegetation to absorb photosynthetically active

radiation (PAR). Therefore, the between-species differences in the spectral indices CHL and NDVI observed in this study indicate that *P. maritimum* generally displayed a higher capacity to capture energy, a main determinant of the productivity of plants systems (Monteith *et al.*, 1977). Although CHL and NDVI have also been positively related to leaf nitrogen content (Gamon *et al.*, 1995; Justice *et al.*, 1998), we did not observe parallel variations in CHL and NDVI values and leaf nitrogen content (in either of the species studied) along the dune gradient - especially at the inland end, where leaf nitrogen content was lower in *P. maritimum* (in which the values for the spectral indices were highest) than in *E. maritimum*.

The effective quantum yield of photosystem II (Φ PSII) measures the proportion of light absorbed by the chlorophyll used in photochemistry, and it has been positively correlated with radiation use efficiency (Genty *et al.*, 1989; Maxwell and Johnson, 2000; Roháček, 2002) but see Wu *et al.*, 2010). Although radiation use efficiency has been reported to differ significantly between species and environmental conditions, as a result of varying environmental constraints (Field *et al.*, 1994; Garbulsky *et al.*, 2010), we found that the Φ PSII values did not differ between species and was not spatially dependent along the shore-to-land gradient. The lack of variability in Φ PSII values suggests that, although the leaves of both species were morphologically different, both types are able to maintain very similar photochemical efficiencies along the whole dune gradient. However, the species differed in PRI values, which, unlike Φ PSII values, exhibited clearly directional spatial dependence along the gradient (increasing steadily from shoreline to inland sites) in *P. maritimum*. Thus, although the results of this study do not support previous reports of close relationships between PRI and Φ PSII values (see review in Garbulsky *et al.*, 2011), they are consistent with other reports of widely varying relationships between PRI and Φ PSII in different species (Peñuelas *et al.*, 1995; Filella *et al.*, 1996; Styliniski *et al.*, 2002; Guo and Trotter, 2004; Busch *et al.*, 2009). PRI values are inversely

related to the dissipation of excess radiation energy as heat, a photoprotective mechanism that involves the xanthophyll cycle in thylakoid membranes (Gamon *et al.*, 1992; Peñuelas *et al.*, 1995; Demmig-Adams *et al.*, 1996; Filella *et al.*, 1996; Gilmore *et al.*, 1996; Gamon *et al.*, 1997; Barták *et al.*, 2004). Therefore, the correlation between PRI and Φ PSII may be weak when the xanthophyll cycle is prepared for sustained thermal dissipation of the light energy absorbed (Busch *et al.*, 2009). PRI values have also been inversely related to the ratio of total carotenoids to chlorophyll contents (Peñuelas *et al.*, 1995; Sims *et al.*, 2002; Stylinski *et al.*, 2002) and the ratio between β -carotenes and chlorophylls (Filella *et al.*, 2009), which may protect the leaves from photoinhibition (Gamon *et al.* 1997). According to Guo and Trotter (2004), interspecies differences in the relationships between PRI and Φ PSII are probably due to differences in the size of the carotenoid pool relative to the size of the total chlorophyll pool. Thus, the lower PRI values in *E. maritimum* than in *P. maritimum*, but similar Φ PSII values, can be explained by a low ratio of carotenoids to chlorophylls in *E. maritimum*. In general, plants with high saturating irradiance and CO₂ uptake rates are expected to have less need for photoprotection and therefore would require a lower ratio of carotenoids to chlorophyll (Guo and Trotter, 2004). The present results suggest that the species under study differed in the way they use light energy and in the requirements for photoprotection, which was greater in *E. maritimum*, as indicated by lower PRI values. The latter species could also cope with the excess radiation by alternative means related to leaf morphology, such as diminishing the leaf surface exposed to incident light by folding and/or light reflection by epicuticular waxes.

Nitrogen is a key element in pigment-protein molecules related to photon capture and transport to photosystems. It is also a component of key enzymes involved in the photosynthetic reactions, such as Rubisco and other related proteins (Evans, 1989; Evans and Poorter, 2001; Wright *et al.*, 2004). The results of the present study did not show any consistent relationship between

soil and leaf nitrogen content and $\delta^{15}\text{N}$ values for any of the species studied. The lack of any relationship between soil N and leaf N may be explained by the fact that soil nitrogen content was determined in soil samples from 25 cm depth, whereas the plants under study may be exploiting soil nutrients at different depths (niche differentiation). Niche differentiation is also a possible explanation for the observed differences in the leaf nitrogen contents of the species studied. This is a novel finding that does not conform to the common observation that coexisting species are similar with respect to the concentrations of mineral nutrients in their leaves (see Grime 2006, and references therein), and suggests that the plants under study may differ in their nitrogen demands, as well as in their allocation to different structures and/or in their ability to take up soil nitrogen. In this study, leaf N was also not related to changes in leaf $\delta^{15}\text{N}$, which is somewhat surprising as several field studies have reported correlations between both leaf traits (Vitousek *et al.*, 1989; Hogberg, 1990; Garten, 1993; Garten *et al.*, 1994; Johannisson *et al.*, 1994; Hobbie *et al.*, 2000; Kitayama *et al.*, 2001; BassiriRad *et al.*, 2003). The species under study only appeared to differ in leaf $\delta^{15}\text{N}$ in the site located 200 m from the shoreline. Differences in the source, absorption and assimilation of nitrogen (Evans and Poorter, 2001) or in the degree of mycorrhizal infection (Handley *et al.*, 1993; Pate *et al.*, 1993; Högberg, 1998; Hobbie *et al.*, 2000) may explain interspecies differences in leaf $\delta^{15}\text{N}$.

Although carbon isotope discrimination ($\Delta^{13}\text{C}$) is a long-term integrator of ecophysiological processes such as leaf conductance and photosynthetic capacity, it has mainly been used as a surrogate for potential water use efficiency (Korner *et al.*, 1988; Farquhar *et al.*, 1989; Ehleringer, 1993). In the present study, the $\Delta^{13}\text{C}$ values suggested a significantly lower WUE in *E. maritimum* than in *P. maritimum* at both ends of the dune gradient, but no significant differences between the species along the rest of the gradient. The water availability for plants is lowest at both ends of the gradient, either because of the scarcity of water in the soil (inland end; see Fig. 4a), or because

water is osmotically retained due to the high soil salinity (shoreline end).

The patterns of variation in $\delta^{15}\text{N}$ and ΦPSII did not show any spatial dependence along the dune gradient for any of the species studied. The other functional traits showed spatial dependence that was only directional for CHL, NDVI and PRI values in *P. maritimum*, thus confirming the existence of a gradient in the responses of this species along the shoreline-inland transect. This also demonstrates a clear divergence in the response strategies of the species under study. However, none of the soil physicochemical variables measured were correlated with these responses along the entire gradient. There was no clear relationship between patterns of variation in functional and structural traits, except for stomatal density in *P. maritimum*, which increased from shoreline to inland sites concurrently with the increases in NDVI and PRI values.

Is the convergence in leaf traits higher at the shoreline end of the dune gradient, where harsher environmental conditions are expected, than at the inland end of the gradient?

The expectation of greater convergence in species traits at the shoreline end of the dune gradient was not supported by the results of this study. Species divergence in functional and structural traits was greater at the shoreline end of the dune gradient than at the inland end in only half of the traits examined. Our expectation of greater convergence in structural and functional traits of species at the shoreline end of the dune gradient was based on previous reports that suggested that stress factors may constrain the range of plant responses in hostile environments, such as coastal sand dunes, thus producing functional convergence of species as predicts the environmental filtering theory (Webb *et al.*, 2002; Reich *et al.*, 2003; Meinzer, 2003; Bucci *et al.*, 2004; Swenson and Enquist, 2007; Jacobsen *et al.*, 2008). However, in environments in which the main stress factors are related to competition rather than to physical factors,

Limiting Similarity has been suggested to be more important in structuring communities, thus leading to trait divergence and consequently niche differentiation (MacArthur and Levins, 1967; Abrams, 1983; Stubbs and Wilson, 2004; Mouillot *et al.*, 2007). However in shoreline sites, the harsh and unpredictable disturbances could also cause divergence in functional traits, according to the disturbance filters proposed by Grime (2006). Other processes such as facilitation and mutualism can lead to either divergence or convergence of traits (Valiente-Banuet *et al.*, 2007; Sargent *et al.*, 2008). Although it is commonly assumed that the severity of the abiotic factors decreases gradually between shoreline and inland sites (Houle, 1996; Griffiths, 2006; Lortie and Cushman, 2007; Kim and Yu, 2009), the existence of comparable levels of convergence in the traits of the species under study, at both ends of the dune gradient, suggests that processes operating in opposite directions, which generate convergence or divergence in traits, may be equally important along the entire gradient. The lack of a consistent directionality in the severity of most of the abiotic stress factors may explain the comparable levels of trait convergence at both ends of the dune gradient. Dune systems represent complex environments where harsh abiotic conditions occur simultaneously with high heterogeneity and unpredictability at multiple spatial and temporal scales. The present results support the idea that there is no optimal configuration of traits for adaptation to these complex environments, and rather that there are multiple suboptimal designs that allow the species to grow together while minimizing competitive interactions.

CONCLUSIONS

Although many studies have assumed that species along dune gradients experience consistent, progressive directional changes in abiotic factors, the results of our study provide clear evidence that some environmental gradients are not perceived as such by species or are perceived as non-directional

variations. Changes in habitat suitability induced by species activity, as well as selection by species of micro-habitats originated by spatial heterogeneity and complex interactions between factors (including mutualistic associations) may disrupt the way that species perceive supposedly directional abiotic gradients. The observed significant differences in structural and functional traits related to the use of resources such as light, water and nutrients, as well as in some characteristics of the soils underneath the plants, strongly suggest that the species studied have evolved different strategies for surviving the harsh environmental conditions in coastal dune systems. Since both species coexist along the whole dune gradient, we conclude that alternative designs of similar fitness might evolve in the same environment. Lack of correspondence between the patterns of variation in leaf traits and soil physicochemical variables allows us to conclude that species rarely respond to the action of single environmental factors. Therefore, claims that plant responses along dune gradients are determined by a single environmental factor are often unfounded. Finally, we conclude that processes that occur along the dune gradient and that induce convergence or divergence in species' traits are of comparable importance.

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Chapter **4**.

A sunny day at the beach: How coastal dune perennial herbs respond to short-term variations in light availability.

SHORT TITLE: Response of dune herbs to short-term variations in light.

KEYWORDS: Chlorophyll fluorescence; coexisting species; dune gradient; functional traits; light-response curves; photochemical efficiency; post-illumination recovery.

ABSTRACT

Light plays an essential role in determining plant structure and functioning in dune ecosystems, which are characterized by high incident and reflected radiation. The variation in this resource demands great plasticity in the photosynthetic system of plants.

In this study, we assessed the phenotypic plasticity of several foredune species by analysing light response curves and dark recovery curves for chlorophyll fluorescence parameters (maximum and effective quantum yield

of photosystem II and photochemical and non-photochemical fluorescence quenching) measured under field conditions. We also addressed how coexisting foredune species with different leaf morphologies and life forms differed in photochemical efficiency in response to short-term changes in light. Finally, we examined how the varying intensity of stressors operating along a dune gradient affected how one of the species (*Pancratium maritimum*) responds to light. The plant responses were examined in winter and summer.

The foredune species differed in their light use strategies but showed similar patterns of dark recovery. Species differences in PSII photochemistry varied seasonally: *Matthiola sinuata* was described as winter specialist and *Eryngium maritimum* as a summer specialist, whereas *Euphorbia paralias* and *P. maritimum* were considered more generalist species, since the photochemical efficiency of both was similar in both seasons. Seasonal differences in the relative performance may allow species to coexist.

Some of the traits used to measure *P. maritimum* photochemistry varied significantly along the dune gradient. Unexpectedly, other traits did not vary as predicted or did not vary at all. For example, changes in PSII efficiency in *P. maritimum* along the gradient were not consistent with assumed directional changes in the severity of stressors. The combination of spatially and temporally variable environmental factors influenced plant performance and determined unpredictable patterns of variation along the gradient in traits measuring PSII photochemistry. The very similar patterns of dark recovery, irrespective of the experimental conditions, suggest that evolutionary pressures may be acting to maintain very similar recovery mechanisms.

INTRODUCTION

Coastal sand dunes are hostile environments in which plant establishment, survival and growth are severely limited by the harshness of the abiotic conditions and highly recurrent natural and human disturbances (Hesp, 1991;

Maun, 2009). The many environmental constraints that plants must cope with in sand dune systems include low availability of soil water and nutrients, high winds, sandblasting, burial by sand, high soil salinity, salt spray deposition and high soil surface temperatures (Chapman, 1976; Watkinson *et al.*, 1978; Payne *et al.*, 1984; Barbour *et al.*, 1985; Maun, 1994; Maun, 2009). Solar radiation also plays an essential role in dune systems, which are characterized by high intensity of incident solar radiation and of radiation reflected by sand surfaces (Barbour *et al.*, 1985; Hesp, 1991; Davy *et al.*, 2006). The lack of a dense overstorey that attenuates radiation in many dune systems, especially in foredunes, may further enhance the effects of high irradiance on dune plants. The essential role of light in plant growth and development is beyond dispute. Light affects plant metabolism directly through photosynthesis, one of the most important biological processes. Therefore, plant growth has been directly related to the plant's ability to intercept solar radiation and convert it into dry matter (Monteith *et al.*, 1977; Woodward, 1987). Apart from its unique role in photosynthesis, light also controls many developmental processes in plants, such as germination, leaf and stem expansion, chlorophyll synthesis, flower induction and phototropism (Fitter *et al.*, 2001). Consequently, light has been considered the single main factor determining ecosystem structure and functioning (Whatley *et al.*, 1980).

In nature, the intensity of light varies greatly both temporally and spatially. Light may vary seasonally, daily, or even within a few seconds as a result of shading by clouds. Large changes in irradiance may also be observed at a small spatial scale, because of differences in the position of a given plant stand within the canopy (Björkman *et al.*, 1995; Battaglia *et al.*, 2002) or because of different exposure due to microtopographical heterogeneity (Stoutjesdijk *et al.*, 1992). Competition for light is also likely to increase along dune gradients (Gilbert *et al.*, 2007). The large variation in this essential resource demands great plasticity, at both large and small scales, in the responsiveness of the photosynthetic system of plants (Björkman and Demmig-Adams, 1995;

Valladares *et al.*, 2000). The correct dose of photosynthetically active radiation (PAR) provides plants with the energy needed for photosynthetic function, which is fundamental to plant metabolism and crucial for the ecological success of plants. At low photosynthetic photon flux density (PPFD), light must be absorbed with maximum efficiency, otherwise it may fall below the compensation point, i.e. the point at which respiration costs are greater than photosynthetic income in plants. On the other hand, an excess of excitation energy may damage photosynthetic reaction centres if light energy absorption exceeds the capacity for light use in photosynthesis (photoinhibitory damage). Although light is of paramount importance for plant life, the responses of dune plants to varying light intensity have been poorly studied at the level of leaf physiology. In particular, very few physiological studies have been carried out under natural conditions, in which the responses of plants to light may be strongly influenced by multiple stress factors, such as water and nutrient deficiency, soil salinity, wind exposure and extreme temperatures (Demmig-Adams *et al.*, 1992; Osmond, 1994; Lichtenthaler *et al.*, 1999; Naumann *et al.*, 2009), all of which occur simultaneously with high quantum flux density in sand dunes. Dune environments are spatially very heterogeneous and some of the above-mentioned stressors may vary widely over relatively short distances (Salzman *et al.*, 1985; Houle, 1997b; Gagne *et al.*, 2001). At larger spatial scales, the intensity of stressors may increase or decrease consistently over long shoreline-inland dune gradients (Kachi *et al.*, 1979; Maruyama *et al.*, 1981; Miura *et al.*, 1983; Hesp, 1991). Generally, the most hostile environmental conditions occur at the coastal end of such gradients because of the greater severity of abiotic factors (e.g. high airborne and soil salinity, low availability of nutrients, burial by sand, and sand blasting) (Ishikawa *et al.*, 1995; Costa *et al.*, 1996; Houle, 1997a; Griffiths, 2006; Lortie *et al.*, 2007; Kim *et al.*, 2009).

Considering that light is one of the main determinants of plant performance in dune systems, we aimed to characterize some aspects of plant

plasticity under natural field conditions, such as their ability to adjust their photosynthetic responses to varying light doses and to regulate the level of excitation energy. We considered plants species that are structurally very distinct but that coexist in a homogeneous foredune area. We first addressed the differences in the responses of these plants to short-term changes in light availability, in terms of photochemical efficiency and the relative importance of the different strategies used to dissipate excess excitation energy. We expected that the differences in leaf anatomy and life forms of the species studied would significantly influence the responses to light. In addition, we examined how the varying intensity of multiple stress factors operating along a shoreline-inland dune gradient influenced light responses in *Pancratium maritimum*, a species that grows along the entire dune gradient. We expected that changes in the severity of stressors along the dune gradient would greatly influence light responses in *P. maritimum*. More specifically, assuming that the severity of the complex of abiotic factors is greater at the shoreline end of the dune gradient, as previously reported (see references above), we predict a lower capacity for light-saturated photosynthesis and slower post illumination recovery in *P. maritimum* plants at this end of the gradient, as result of excessive light energy. The combination of environmental factors that limit a plant's capacity for light saturated photosynthesis and recovery (e.g. low temperatures in winter and high temperatures and water deficits in summer) may change substantially over time, and therefore we also examined how plant responses, in terms of photochemical performance, were affected by winter and summer conditions. As far as we know, no previous studies have addressed these important issues under natural field conditions, in which plant responses to light may be strongly influenced by multiple interactions with other factors. Plant phenotypic plasticity in response to short-term variations in the light dose was examined by analysis of light responses curves and recovery curves after illumination of chlorophyll fluorescence parameters. Light curves allow exploration of species differences in response to light over

a wide range of PPFs. On the basis of the principle of pulse amplitude modulation, chlorophyll fluorescence parameters provide information about qualitative and quantitative changes in the efficiency of photosystem II (PSII) photochemistry (Maxwell *et al.*, 2000). PSII is recognised as the part of the photosynthetic apparatus that is most vulnerable to light-induced damage (Björkman and Demmig-Adams, 1995). Therefore, damage to PSII will often be the first manifestation of stress in a leaf. To analyse the ability of the plants to regulate the level of excitation energy, we determined the relative amount of light channelled through photochemical processes and through alternative means of non-photochemical dissipation, such as emission of fluorescence, mainly from PSII chlorophyll *a*, and non-radiative dissipation of energy into heat.

MATERIALS AND METHODS

Species and location

Four perennial dune herbs with contrasting leaf morphologies and life forms were selected for study. *Eryngium maritimum* L. (Umbelliferae) is a C₃ hemicryptophyte with blue-green three-lobed folded leaves. The leaves are also stiff, leathery and spiny. *Pancreatium maritimum* L. (Amaryllidaceae) is a C₃ cryptophyte with a bulbous stem from which a long glaucous neck grows, ending in long thick linear-shaped leaves of up to 50 cm in length. *Matthiola sinuata* (L.) R. Br. (Brassicaceae) is a hemicryptophyte with a basal rosette of narrow highly pubescent pale grey-green leaves. The plants grow to up to 40 cm in height and the leaves up to 10 cm in length. *Euphorbia paralias* L. (Euphorbiaceae) is a glaucous herb with both fertile and sterile branches growing from a woody base and with closely overlapping leaves. The leaves, of mean size 20 mm, are thick and ovate to elliptic; white latex emanates when the leaves are excised. This plant is a C₃ chamaephyte, although under certain

environmental conditions can switch facultatively to C₄ or CAM metabolism (Elhaak *et al.*, 1997; Daniela *et al.*, 2009).

Two coastal dune systems in Galicia (NW Spain) were selected according to the objectives of the study. For interspecific comparisons, a homogeneous flat area of about 220 m² was selected in the foredune of the Lariño coastal dune system (42°46'00"N, 9°06'58" W; Galicia, NW Spain), where all the above species were interspersed. To study how the varying environmental conditions that occur along dune gradients influence the photochemical efficiency of *P. maritimum*, we selected a 800 m long coast-inland gradient in the coastal dune system of Nerga (42°15'50"N, 8°51'10"W; Galicia, NW of Spain), where *P. maritimum* grows from shoreline to inland locations.

Chlorophyll fluorescence measurements

Light and dark-adapted chlorophyll fluorescence parameters were measured in the field with a portable pulse amplitude modulated fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany) attached to an Arabidopsis Leaf Clip Holder 2060-B (Heinz Walz GmbH, Effeltrich, Germany). The incident photosynthetic photon flux density (PPFD) on leaves was supplied by an external halogen lamp (2050-HB, Heinz Walz GmbH, Effeltrich, Germany) coupled to the fluorometer. Minimum (F_0) and maximum (F_m) fluorescence emissions were measured under dark adapted conditions (Maxwell and Johnson, 2000; Roháček, 2002). The steady state or basal fluorescence emission (F_i) and the maximum emission of fluorescence (F'_m) were determined in light-adapted samples (Maxwell and Johnson, 2000; Roháček, 2002). F_m and F'_m were measured after a light saturating pulse ($> 4000 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 0.8 s pulse length) of actinic white light (Maxwell and Johnson, 2000; Roháček, 2002). These basic chlorophyll fluorescence parameters were used to compute different indices, as follows. The effective quantum yield of photosystem II was determined as $\Phi\text{PSII} =$

$(F'_m - F_t) / F'_m$ (Genty *et al.*, 1989). This index, which measures the fraction of the light absorbed by chlorophyll (associated with photosystem II) that is used in photochemistry, has been inversely correlated with the dissipation of excess radiation energy as fluorescence and is directly correlated with radiation use efficiency (Genty *et al.*, 1989; Maxwell and Johnson, 2000; Roháček, 2002). The maximum quantum yield of photosystem II (F_v / F_m) was calculated from the measured values of F_m and F_0 , after a saturation pulse, as $(F_m - F_0) / F_m$. This was determined in dark adapted samples with all reaction centres of photosystem II fully open (i.e. all primary acceptors oxidized). The F_v / F_m ratio estimates the efficiency with which the excitation energy is captured by open photosystem II reaction centres and represents the proportion of incident photon energy that is processed photochemically (Krause *et al.*, 1991; Mohammed *et al.*, 1995). In healthy leaves of most species, the value of F_v / F_m is about 0.8 (Krause and Weis, 1991; Maxwell and Johnson, 2000). F_v / F_m values below 0.8 have been considered symptomatic of stress-dependent photoinhibition (Bjorkman *et al.*, 1987; Long *et al.*, 1994; Maxwell and Johnson, 2000). The amount of chlorophyll fluorescence quenched photochemically was assessed by the photochemical quenching index (qP), calculated as $(F'_m - F_t) / (F'_m - F_0)$ (Maxwell and Johnson, 2000; Roháček, 2002). The qP index estimates the proportion of PSII reaction centres that are open, and it therefore estimates the availability of these reaction centres to process photon energy photochemically (Krause and Weis, 1991; Maxwell and Johnson, 2000; Roháček, 2002). Finally, the non-photochemical quenching of chlorophyll fluorescence was calculated by the qN index, as $1 - ((F'_m - F_0) / (F_m - F_0))$ (Maxwell and Johnson, 2000; Roháček, 2002). The qN index indicates the level of non-photochemical energy dissipation in the PSII light-harvesting antenna relative to the dark-adapted state (Maxwell and Johnson, 2000). It is commonly assumed that the reduction of fluorescence by non-photochemical processes is caused by the loss of photon energy *via* thermal dissipation (Björkman and Demmig-Adams, 1995; Buschmann, 1999; Roháček, 2002).

Non-photochemical quenching is induced by changes in the pH gradient at the thylakoid membrane, by state transitions and by photoinhibitory processes (Krause *et al.*, 1982; Horton *et al.*, 1988; Bilger *et al.*, 1990; Krause and Weis, 1991; Pospíšil, 1997; Roháček, 2002).

Measurement protocol

In February and August 2008, chlorophyll fluorescence parameters were measured in one fully expanded leaf on each of five randomly selected plants of each species in the Lariño dune system. At the Nerga site, chlorophyll fluorescence parameters were measured, in April and June 2008, in one fully expanded leaf on each of five randomly selected *P. maritimum* individuals, at each of four locations (0, 150, 500 and 800 m) along a shoreline-inland dune gradient. The data required to construct light response curves for each of the selected plants were recorded as follows. The plants were covered with a portable shading structure opaque to photosynthetic active radiation (PAR). After 30 minutes of dark adaptation, parameters F_0 and F_m were measured. Following the initial dark measurements, the external halogen lamp was switched on and a sequence of F_t and F'_m measurements were made at increasing levels of photosynthetic photon flux density (PPFD; 50, 100, 200, 350, 600, 1000 and 1500 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). The light-adapted parameters F_t and F'_m were measured after a prior adaptation period of three minutes to the current PPFD. After recording the final F_t and F'_m measurements at the highest light intensity, the halogen lamp was switched off and plant recovery under dark conditions was recorded, as follows. At the Lariño site, F_0 and F_m were measured after 20 minutes of dark adaptation. At the Nerga site, a recovery curve was constructed with F_0 and F_m data measured after 1, 5 and 20 minutes of dark adaptation. F_0 and F_m , which were measured after the initial 30 minutes of dark adaptation, and F_t and F'_m , which were measured after the 3 minutes adaptation to the highest light intensity (1500 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), were used to determine the maximum and effective

quantum yield of photosystem II and also the photochemical (qP) and non-photochemical (qN) quenching coefficients.

Statistical analysis

Prior to analyses, all variables were checked for the normality and homoscedasticity assumed for Analysis of Variance (ANOVA). No variable severely deviated from the underlying assumptions, thus justifying the use of ANOVA (Zar, 1984). Differences in chlorophyll fluorescence parameters were analyzed by two way analysis of variance, with Species and Season as fixed factors in the interspecific analysis and Species and Position in the gradient analysis. The following functions, both with a type III error structure, were used: the “aov” function of the “stats” package (R Development Core Team, 2009) and the “Anova” function of the “car” package (Fox, 2009). Light response curves were fitted to a non-linear mixed model (Peek *et al.*, 2002; Ritchie, 2008) by the method of random slopes. PPFD was included as a mixed factor dependent on plant species, considering both single and quadratic effects. Stepwise analysis fitting was carried out, with the “lme” function (package “nlme”; Pinheiro *et al.*, 2009) and “anova” function (package “stats”; R Development Core Team, 2009), to calculate the random effect in mixed models, as described by Crawley (2007). Recovery curves constructed with data obtained along the dune gradient were fitted, with the “lme” function, to a nonlinear mixed model by the method of random slopes, including single and cubic effects of time. Recovery lines from interspecific comparisons were fitted, with the “lme” and “anova” functions, to a linear mixed model.

RESULTS

Light responses in coexisting species

Photochemical efficiencies

Species differences in maximum quantum yield of photosystem II were significantly influenced by the factor Season (Table 1). For *M. sinuata*, the F_v / F_m values were significantly lower in summer than in winter (Fig. 1a), and the opposite was found for *E. maritimum*, in which the winter F_v / F_m values were the lowest recorded among all species. However, in *E. paralias* and *P. maritimum*, the F_v / F_m values did not differ significantly between seasons.

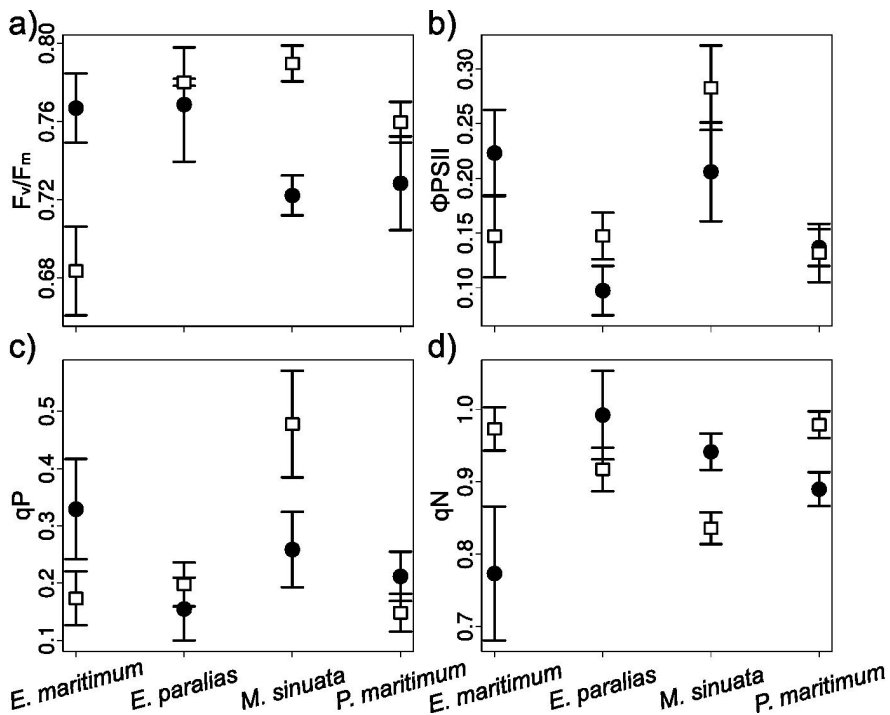


Fig. 1. Mean values (\pm S.E., $n = 5$ plants), in winter (\square) and summer (\bullet), of the chlorophyll fluorescence parameters: a) maximum (F_v / F_m) and b) effective (Φ_{PSII}) quantum yield of photosystem II, and c) photochemical (qP) and d) non-photochemical (qN) quenching of chlorophyll fluorescence, for the species coexisting in the Lariño dune system (*Eryngium maritimum*, *Euphorbia paralias*, *Matthiola sinuata* and *Pancreatium maritimum*).

Table 1. Results of the two way analysis of variance, with Species and Season as fixed factors, for the comparison of the chlorophyll fluorescence parameters, maximum (F_v / F_m) and effective (Φ_{PSII}) quantum yield of photosystem II, and photochemical (qP) and non-photochemical (qN) quenching of chlorophyll fluorescence in the four species coexisting in the Lariño dune system.

df	F_v / F_m			Φ_{PSII}			qP			qN			
	SS	F	P	SS	F	P	SS	F	P	SS	F	P	
Species	3	0.013	1.71	0.185	0.093	3.75	0.020	0.241	2.72	0.061	0.044	0.93	0.439
Season	1	0.0004	0.18	0.674	0.0013	0.16	0.693	0.0011	0.04	0.845	0.007	0.46	0.501
Species × Season	3	0.031	4.14	0.014	0.034	1.38	0.267	0.194	2.19	0.109	0.155	3.27	0.034
Residuals	32	0.080		0.264			0.947				0.504		

Under light conditions ($1500 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), species differed significantly in the effective quantum yield of photosystem II (Table 1); the photochemical efficiencies were lowest in *E. paralias* and *P. maritimum* and highest in *M. sinuata* (Fig. 1b). Differences in the ability of species to dissipate the excess excitation energy by thermal processes, as expressed by the qN index, depended significantly on season (Table 1). Although there were no differences between summer and winter values of qN in *E. paralias*, the values were higher in winter than in summer in *P. maritimum* and, especially, *E. maritimum* (Fig. 1d). Conversely, in *M. sinuata* the amount of absorbed light energy that is dissipated as heat was higher in summer than in winter (Table 1; Fig. 1d). The analyses did not detect any significant effects on the photochemical fluorescence quenching (qP; Table 1; Fig. 1c).

Light curves and post-illumination recovery

The statistical analysis of the light response curves showed significant contributions to the model by introducing the factor Species ($P = 0.0014$) and the Species \times Season interaction ($P = 0.001$). For all species and both seasons, the quantum yield of photosystem II decreased with increasing PPFDs (Fig. 2). In winter, at light intensities above $100 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the photochemical efficiency of *M. sinuata* was significantly higher than that of the other species, which did not differ from each other. However, in summer, at light intensities between 50 and $1500 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the photochemical efficiency of *E. paralias* was significantly lower than that of the other species, which did not differ from each other. Addition of the interactive component (Species by Season) to the model of the post-illumination recovery yielded a statistically significant effect ($P = 0.0067$; Fig. 3). In winter, the measurements made just after the light was switched off showed that the photochemical efficiency was very similar in all species, with values below 0.2, except *M. sinuata*, in which the values were slightly lower than 0.3. After a 20 min period of dark recovery, the photochemical efficiency

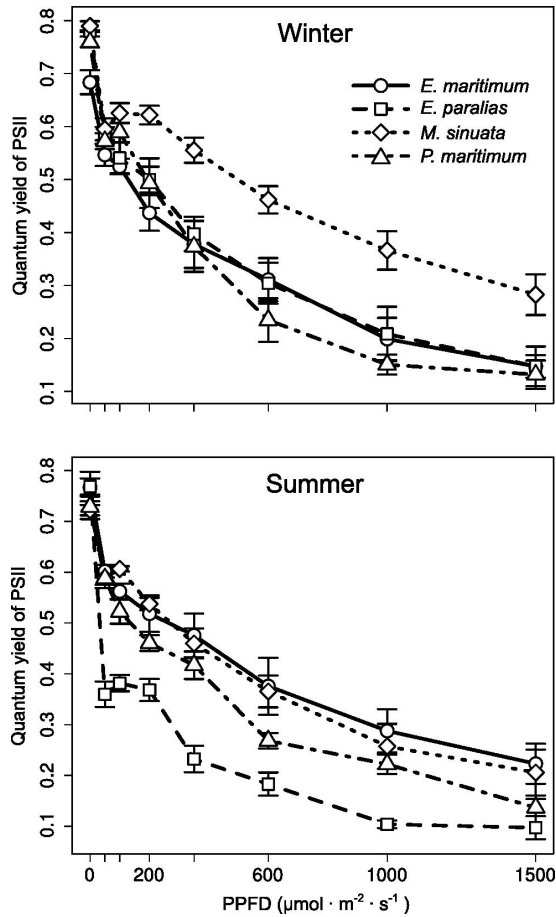


Fig. 2. Light response curves for Winter and Summer, constructed with data measured via the quantum yield of photosystem II, as a function of the quantum flux density (PPFD), for the species coexisting in the Lariño dune system (*Eryngium maritimum*, *Euphorbia paralias*, *Matthiola sinuata* and *Pancreatium maritimum*). Symbols represent mean values of quantum yield of photosystem II (\pm S.E., $n = 5$ plants) after 3 min illumination at the corresponding light intensity, except for the first point at $0 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, which was measured after a 30 min of dark adaptation. Response curves were fitted to a non-linear mixed model by the method of random slopes. PPFD was included as a mixed factor dependent on plant, considering both single and quadratic effects. Stepwise analysis fitting, with the “lme” function (package “nlme”; Pinheiro *et al.*, 2009) and “anova” function (package “stats”; (R Development Core Team, 2009), was used to calculate the random effect in mixed models.

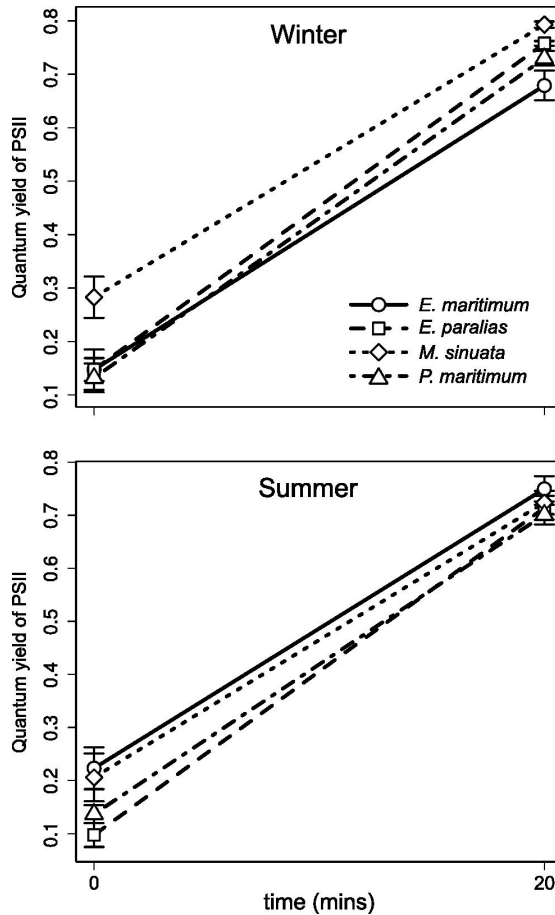


Fig. 3. Quantum yield of photosystem II, in Winter and Summer, as a function of dark recovery time, for the species coexisting in the Lariño dune system (*Eryngium maritimum*, *Euphorbia paralias*, *Matthiola sinuata* and *Pancretium maritimum*). Symbols represent mean values of quantum yield of photosystem II (\pm S.E., $n = 5$ plants) measured after 20 min of dark adaptation. The first point in all graphs represent values of quantum yield of photosystem II measured when the halogen lamp was switched off after the last measurement at the highest light intensity recorded. Recovery lines were fitted, with the “lme” and “anova” functions, to a linear mixed model.

Table 2. Results of the two way analysis of variance, with Species and Position as fixed factors, for the comparison of the chlorophyll fluorescence parameters, maximum (F_v / F_m) and effective (Φ_{PSII}) quantum yield of photosystem II, and photochemical (qP) and non-photochemical (qN) quenching of chlorophyll fluorescence in *Panocratium maritimum* plants growing at four positions along the shoreline-inland gradient in the Nerga dune system.

df	F_v / F_m			Φ_{PSII}			qP			qN			
	SS	F	P	SS	F	P	SS	F	P	SS	F	P	
Position	3	0.0024	0.56	0.641	0.037	3.50	0.027	0.080	0.50	0.687	0.009	2.03	0.130
Season	1	0.012	8.59	0.006	0.004	1.26	0.270	0.130	2.44	0.128	0.005	3.41	0.074
Position \times Season	3	0.0015	0.35	0.784	0.018	1.70	0.187	0.315	1.96	0.140	0.026	5.67	0.003
Residuals	32	0.045			0.112			1.715			0.048		

increased to about 0.7, with *M. sinuata* again showing the highest values, slightly below 0.8, and *E. maritimum* showing the lowest values, slightly below 0.7. In summer, at the beginning of the dark recovery period, the species differed slightly in photochemical efficiency, with values of about 0.2 for *E. maritimum* and *M. sinuata*, and of about 0.1 for *P. maritimum* and *E. paralias*. At the end of the recovery period, the photochemical efficiency of all species converged to very similar values, of about 0.7.

Light responses of P. maritimum along a dune gradient

Photochemical efficiency

The maximum quantum yield of photosystem II in *P. maritimum* did not vary significantly between the different positions along the dune gradient and was consistently significantly higher in summer than in winter (Table 2; Fig. 4a). The effective quantum yield of photosystem II changed significantly with the position along the dune gradient (Table 2; Fig. 4b), decreasing from the coastal end to the 100 m position and increasing from there until the inland end of the gradient. Although neither Season nor the Season \times Position interaction had significant effects (Table 2), the photochemical efficiency of *P. maritimum* was clearly higher in summer than in winter at the inland end of the gradient (Fig. 4b). The photochemical quenching of chlorophyll fluorescence (qP coefficient) was not influenced by Position or Season (Table 2; Fig. 4c). However, changes in the non-photochemical quenching of chlorophyll fluorescence (qN coefficient) along the dune gradient differed significantly between summer and winter (Table 2; Fig. 4d). In winter, qN increased consistently from the coastal to the inland end of the gradient, and the opposite trend was observed in summer. Thus, qN was higher in summer than in winter at the shoreline but lower at the inland positions (Table 2; Fig. 4d).

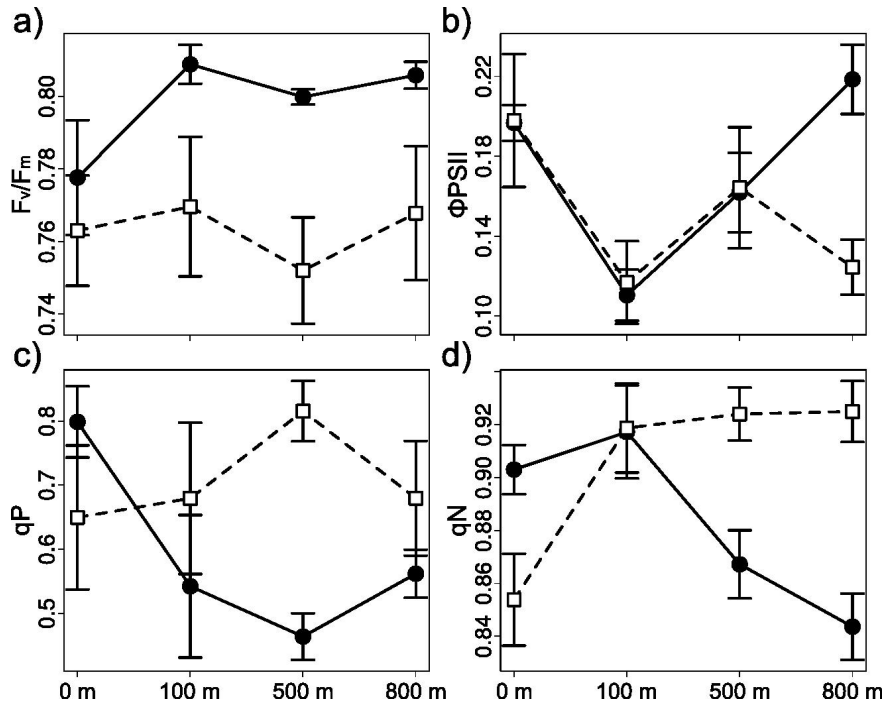


Fig. 4. Mean values (\pm S.E., $n = 5$ plants), in winter (\square and dashed line) and summer (\bullet and solid line), for the chlorophyll fluorescence parameters: a) maximum (F_v / F_m) and b) effective (Φ_{PSII}) quantum yield of photosystem II, and c) photochemical (qP) and d) non-photochemical (qN) quenching of chlorophyll fluorescence, for *Pancratium maritimum* plants growing at four positions (0, 100, 500 and 800 m) along a shoreline-inland gradient in the Nerga dune system.

Light curves and post-illumination recovery

The statistical analyses revealed that the effect of Season ($P = 0.0068$) and the interactive effect of Position by Season ($P = 0.0172$) contributed significantly to the model of the responses of *P. maritimum* to light (Fig. 5). For all positions and both seasons, the photochemical efficiency of *P. maritimum* plants decreased with increasing intensity of PPFDs. In summer, differences in light response curves emerged at radiation fluxes higher than

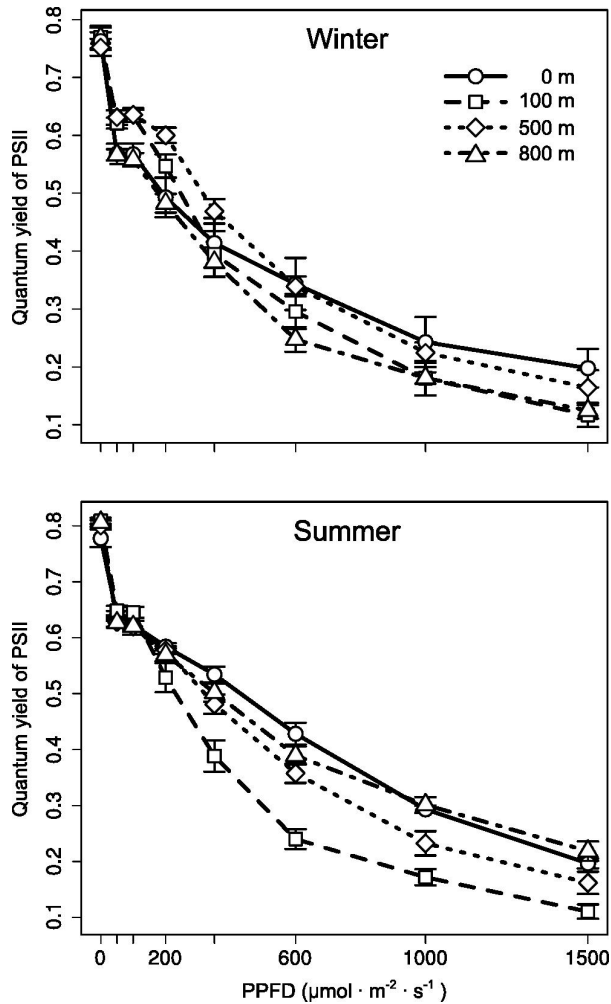


Fig. 5. Light response curves in winter and summer measured via the quantum yield of photosystem II, as a function of the photosynthetic photon flux density (PPFD), for *Pancratium maritimum* plants growing at four positions (0, 100, 500 and 800 m) along a shoreline-inland gradient in the Nerga dune system. Symbols represent mean values of quantum yield of photosystem II (\pm S.E., $n = 5$ plants) after 3 min illumination at the corresponding light intensity, except for the first point at $0 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, which was measured after 30 min of dark adaptation. Response curves were fitted to a non-linear mixed model by the method of random slopes. PPFD was included as a mixed factor dependent on plant, considering both single and quadratic effects. Stepwise analysis fitting, with the “lme” function (package “nlme”; Pinheiro *et al.*, 2009) and “anova” function (package “stats”; R Development Core Team, 2009), was used to calculate the random effect in mixed models.

200 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; for *P. maritimum* plants growing in middle positions of the gradient, especially those at 100 m far from the shoreline, the quantum yields of photosystem II were lower than in plants growing at both extremes of the gradient. Differences in light response curves in winter were only apparent at light intensities equal to or above 50 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Fig. 5). For the post-illumination recovery curves, Position ($P = 0.0021$) was the only factor that significantly contributed to the model. Differences in recovery curves (Fig. 6) were only perceptible in winter after five minutes of dark adaptation: at this time, the photochemical efficiency of *P. maritimum* plants growing at the shoreline was lower than that of plants growing at the other positions. However, by the end of the recovery period, the photochemical efficiency of all *P. maritimum* plants, irrespective of their position along the gradients, was very similar.

DISCUSSION

Light responses and post-illumination recovery in coexisting species

The four species studied, which coexist in a homogeneous area of a dune system, showed different light use strategies but quite similar patterns of post-illumination recovery. For most of the functional traits studied, species differences varied with the season. In *M. sinuata*, the seasonal values for the maximum quantum yield of photosystem II (F_v / F_m) differed markedly, although this parameter has been reported to be seasonally stable (Adams III *et al.*, 2004). In this species, the winter values of this parameter, which represents the maximum efficiency at which light absorbed by PSII pigments is converted to chemical energy (Butler, 1978; Baker *et al.*, 2004), were highest and the summer values were lowest among all species. Considering that F_v / F_m values below 0.75-0.80 are symptomatic of stress-dependent photoinhibition (Bjorkman and Demmig, 1987), the results of this study

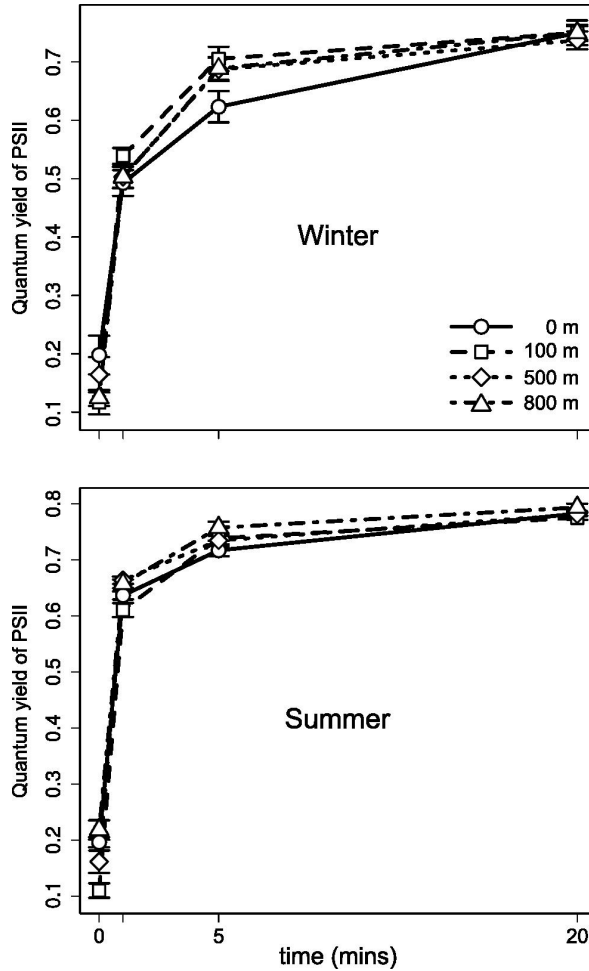


Fig. 6. Quantum yield of photosystem II, in winter and summer, as a function of dark recovery time, for *Pancratium maritimum* plants growing at four positions (0, 100, 500 and 800 m) along a shoreline-inland gradient in the Nerga dune system. Symbols represent mean values of quantum yield of photosystem II (\pm S.E., $n = 5$ plants) measured after 1, 5, and 20 minutes of dark adaptation. The first point in all graphs corresponds to quantum yield of photosystem II values measured when the halogen lamp was switched off after the last measurement at the highest light intensity recorded for the light curves. Recovery curves obtained were fitted to a nonlinear mixed model, including single and cubic effects of time, by the method of random slopes (with the “lme” and “anova” functions).

strongly suggest that *M. sinuata* is more susceptible than *E. maritimum* and *E. paralias* to photoinhibition induced by summer stress conditions, but that it is more tolerant than all of the other species to winter conditions that could cause photoinhibition (although see below on photoinhibition susceptibilities). The values of the effective quantum yield of photosystem II, Φ_{PSII} , a measure of the proportion of light absorbed by chlorophyll that is used in PSII photochemistry in light-acclimated plants (Genty *et al.*, 1989), indicated that in winter, radiation use efficiency is highest in *M. sinuata*. This result is consistent with the low non-photochemical quenching (qN) values measured in *M. sinuata* in winter. The qN values reflect the extent of activation of the PSII complexes of non-photochemical processes that lead to the dissipation of the excess excitation energy as heat (Roháček *et al.*, 2008), which occurs in light-acclimated plants when their capacity for photochemical energy conversion is limited (Genty *et al.*, 1989; Grant *et al.*, 2010). The dense pubescence of *M. sinuata* may provide protection against light stress (Ripley *et al.*, 1999; Liakopoulos *et al.*, 2006; Skelton *et al.*, 2012) and may therefore contribute to explaining why *M. sinuata* is capable of maintaining a high level of photochemical efficiency in winter. These results are consistent with the light response curves, which reveal that *M. sinuata* differs from the other species and is generally the most efficient in the use of light in winter, over the range of PPFDs examined. Results from the post-illumination recovery suggest that species did not differ in their susceptibility to photoinhibition as after 20 minutes of dark acclimation, the maximum quantum yield of photosystem II of all species increased to values representative of dark-adapted, healthy, unstressed leaves. All species recovered at similar rates, as shown by their similar slopes in the recovery lines. Under natural conditions, dune plants may experience longer periods of exposure to higher PPFDs than those used in this study, and therefore we cannot rule out the existence of species differences as regards susceptibility to photoinhibition.

The strategy used by *E. maritimum* was quite different from that used by

M. sinuata. Like *M. sinuata*, in *E. maritimum* the seasonal values for the maximum quantum yield of photosystem II and for the non-photochemical quenching of chlorophyll fluorescence, expressed by the qN coefficient, were very different. However, unlike *M. sinuata*, *E. maritimum* makes better use (than all of the other species) of the amount of light in summer. In summer, *E. maritimum* exhibited the highest effective and maximum quantum yields of photosystem II of all species. It is also the only species in which photochemical efficiency was higher in summer than in winter. These results are consistent with summer qN values in *E. maritimum*, which were the lowest among all the species, suggesting that in this species the non photochemical processes leading to the dissipation of the excess excitation energy as heat are less active than in the other species, because of its greater photochemical efficiency. The present findings are consistent with those of Anderson *et al.* (2011), who reported summer F_v / F_m values for *E. maritimum* in the range 0.77-0.83. Light curves also confirm that the relative photochemical performance of *E. maritimum* is higher in summer, and that the efficiency was highest over the range of PPFs examined, especially at higher light intensity, when light utilization is distinctly restricted in the different species. The particular leaf morphology of this species (thick waxy cuticle, folded leaves) may contribute to minimizing the intensity of incident sunlight and diminishing the amount of excess energy in summer (Williams *et al.*, 2003).

The strategy of *E. paralias* is different from those described for *M. sinuata* and *E. maritimum*, in which the seasonal differences in various aspects of the photochemistry of photosystem II were very different. The photochemical performance of *E. paralias* was similar in summer and winter. Elevated values of the maximum quantum yield of photosystem II, both in summer and in winter, indicate that this species is tolerant to a wide range of conditions that could induce photoinhibition. However, light-acclimatized plants of this species showed low levels of light use efficiency, especially in summer, as expressed by the lowest effective quantum yields of photosystem

II among all species. This result is quite surprising considering that under certain environmental conditions, *E. paralias* can switch facultatively to C₄ or CAM metabolism (Elhaak *et al.*, 1997; Daniela *et al.*, 2009). The highest summer qN values registered in this species suggest that heat dissipative processes may be very important for regulation and protection of photosystems in environments where light energy absorption exceeds the capacity for light utilization in photosynthesis. This interpretation conforms with the low PSII photochemical efficiencies recorded. The light response curves also confirm that in this species the light use efficiency was very low over the PPFDs considered. This was particularly obvious in the light response curves recorded in summer, when *E. paralias* showed the lowest PSII efficiency of all the species. Differences were apparent even at low light intensity.

We found that *P. maritimum* adopts a strategy somewhat similar to that described for *E. paralias*. Both species maintained similar seasonal values for the maximum and the effective quantum yields of photosystem II. However, the winter performance of *P. maritimum* in terms of maximum quantum yield of photosystem II appeared to be slightly poorer than that of *E. paralias*. The highest winter qN values recorded in *P. maritimum* denote the high activity in this species of non-photochemical processes of dissipation of the excess of excitation energy as heat, which is consistent with the low PSII efficiency in light-acclimatized plants. These results are consistent with the information provided by light curves, which showed the low winter efficiency of the PSII photochemistry in *P. maritimum* plants at light intensities higher than 350 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

We acknowledge that these results may somewhat underestimate photochemical efficiencies in *P. maritimum*. In plants such as *P. maritimum*, with thick leaves and multiple layers of overlapping cells, chlorophyll fluorescence techniques may only characterize the upper chloroplasts of the external palisade parenchyma cell layers and may thus underestimate the

global efficiency of the full leaf (Lichtenthaler and Burkart, 1999).

Light responses and post-illumination recovery of *P. maritimum* along a dune gradient

As expected, we found that some functional traits related to light processing efficiency through PSII varied significantly in *P. maritimum* plants located along a shoreline-inland dune gradient. However, unexpectedly, other functional traits did not vary as predicted or did not vary at all. Community assembly studies suggest that in harsh environments, abiotic factors may function as environmental filters that constrain the range of plant physiological responses (Reich *et al.*, 1997; Paruelo *et al.*, 1998; Meinzer, 2003; Bucci *et al.*, 2004). On the basis of these results, we expected that the greater severity of stressors at the shoreline end of the dune gradient may constrain a plant's capacity for light-saturated photosynthesis and thus induce photoinhibitory PSII damage due to an increased level of excitation energy. Nevertheless, the high F_v / F_m values measured along the whole dune gradient suggest that there was no irreversible damage to photosystem II of *P. maritimum* at any of the positions along the gradient. A decrease in the values of F_v / F_m , the maximum quantum yield of photosystem II photochemistry, has been extensively used to assess the extent of the irreversible inhibition of PSII (Maxwell and Johnson, 2000). On the other hand, in light-acclimated plants, the effective quantum yield of photosystem II photochemistry varied significantly along the dune gradient. Although we expected the lowest PSII efficiencies in plants growing at the shoreline, the lowest efficiencies were actually observed in *P. maritimum* plants growing 100 m from the shoreline. In a previous study in which we examined changes in physicochemical soil variables along a dune gradient, the lowest amounts of soil nitrogen and phosphorous and the highest soil salinity were recorded at some distance from the shoreline (chapter 3; submitted). It is possible that airborne salt deposition (McLachlan *et al.*, 2006) may be higher immediately behind the foredune, as a

result of the wind turbulence caused by the dune ridge, and that this may adversely affect plant performance. These findings suggest that for some species, such as *P. maritimum*, stressful environmental conditions limiting the plant's capacity to process radiation efficiently may not only occur at the shoreline end of the gradient. Other results support this interpretation. In this study, in both seasons, the highest values of the non-photochemical quenching of chlorophyll fluorescence (qN) were recorded in *P. maritimum* plants located at 100 m from the shoreline, although in winter, the qN values were similar for the three positions further inland. Since the amount of non-photochemical quenching is considered an indicator of the severity of light stress, specifically of the excess absorbed light (Buschmann, 1999), these findings suggest that at a distance of 100 m from the coast, stress factors may restrict the capacity of photosynthetic apparatus of *P. maritimum* plants to use the total amount of absorbed light energy for photochemistry. Under such conditions, non-photochemical quenching is induced to dissipate the excess excitation energy by thermal processes, precluding over-reduction of the electron transfer chain and, therefore, providing protection from light-induced damage (Buschmann, 1999). Further results from this study reinforce the interpretation of more severe environmental conditions at a certain distance from the shoreline. The light response curves constructed with data recorded in summer revealed a more pronounced decline in PSII efficiency with increased PPFD in *P. maritimum* plants growing at a distance of 100 m from the shoreline. However, dark-recovery curves constructed with data recorded in winter revealed a slower pattern of recovery in the quantum yield of photosystem II in *P. maritimum* plants growing at the shoreline. Nevertheless, at the end of the dark-recovery period, very similar F_v / F_m values were reached in all *P. maritimum* plants, irrespective of their position along the gradient. The values were representative of a high quantum yield of photosystem II, indicating that the plants did not exhibit different susceptibilities to irreversible light-induced damage to PSII reaction centres. An alternative, plausible explanation of the

differences observed in PSII efficiencies of *P. maritimum* is that local adaptation processes may have originated an ecotype specially adapted to the local conditions of the shoreline, with improved efficiencies compensating the more severe abiotic conditions existing at this extreme of the dune gradient. Previous studies support this interpretation. Crawford (1989) reported the occurrence of different ecotypes of *Plantago maritima* along an 80 m transect of cliff top vegetation, and Hodson *et al.* (1982) described a salt-tolerant coastal ecotype and a salt-susceptible inland ecotype of *Agrostis stolonifera*.

In conclusion, this study demonstrates that the coexisting species under study, growing in a homogeneous area of a harsh dune system, clearly diverge in various aspects of their photochemistry, indicating different strategies related to the light processing efficiency through photosystems. In relation to the different light use strategies, *M. sinuata* can be described as a winter specialist and *E. maritimum* as a summer specialist, whereas *E. paralias* and *P. maritimum* could be considered as more generalist species, since both were capable of maintaining similar photochemical efficiency in both seasons, although *E. paralias* displayed more efficient light use in winter and *P. maritimum* in summer. Although these differences conform to our expectations based on the observed differences in leaf morphologies and life forms of the species studied, they contrast with the predictions, often made in community assembly studies, that in harsh environments, such as dune systems, stress factors may act as environmental filters that constrain the range of possible functional designs. The seasonal differences in the functional traits of the species suggest that the relative performance of species in terms of photochemical efficiencies varies over time. When environmental conditions vary temporally and the relative performances of species also change, environmental variation may allow species to coexist where otherwise (i.e. under constant conditions) one species would exclude the other.

The photochemical efficiency of *P. maritimum* shifts along the dune gradient. Although many studies have reported that dune gradients are

directional, with the intensity of stressors increasing or decreasing consistently along the dune gradients, this study provides evidence that changes along the dune gradient in some leaf functional traits in *P. maritimum*. The findings indicate that the efficiency of PSII photochemistry and heat dissipation were not consistent with the assumed directional changes in the severity of the stressors. The study also demonstrated that some of these changes were seasonally dependent. From all this, we conclude that the combination of environmental factors that influence plant performance, which vary spatially and temporally, may determine unpredictable patterns of variation along the dune gradient in leaf traits related to the functioning of various aspects of PSII photochemistry.

Finally, we found that, despite the large variation in leaf functional traits related to photochemistry capacity and to the extent of dissipative mechanisms of excitation energy excess, the plants followed very similar patterns of post-illumination recovery, irrespective of the species, the season, the set of environmental conditions studied or whether different species were growing in a homogeneous area or a single species was growing along a dune gradient. This suggests that evolutionary pressures may be acting to maintain very similar recovery mechanisms.

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Chapter 5.

Soil water content and patterns of allocation to below- and above-ground biomass in the sexes of the subdioecious plant *Honckenya peploides*.

SHORT TITLE: Sexual differences in seasonal patterns in *H. peploides*.

KEYWORDS: dioecy, biomass allocation, below-ground structures, reproductive effort, spatial segregation, water availability.

ABSTRACT

Background and aims Dioecious plants often show sex-specific differences in growth and biomass allocation. These differences have been explained as a consequence of the different reproductive functions performed by the sexes. Empirical evidence strongly supports a greater reproductive investment in females. Sex differences in allocation may determine the performance of each sex in different habitats and therefore might explain the spatial segregation of the sexes described in many dimorphic plants. Here, an investigation was made of the sexual dimorphism in seasonal patterns of

biomass allocation in the subdioecious perennial herb *Honckenya peploides*, a species that grows in embryo dunes (i.e. the youngest coastal dune formation) and displays spatial segregation of the sexes at the studied site. The water content in the soil of the male- and female-plant habitats at different times throughout the season was also examined.

Methods The seasonal patterns of soil-water availability and biomass allocation were compared in two consecutive years in male and female *H. peploides* plants by collecting soil and plant samples in natural populations. Vertical profiles of below-ground biomass and water content were studied by sampling soil in male- and female-plant habitats at different soil depths.

Key Results The sexes of *H. peploides* differed in their seasonal patterns of biomass allocation to reproduction. Males invested twice as much in reproduction than females early in the season, but sexual differences became reversed as the season progressed. No differences were found in above-ground biomass between the sexes, but the allocation of biomass to below-ground structures varied differently in depth for males and females, with females usually having greater below-ground biomass than males. In addition, male and female plants of *H. peploides* had different water-content profiles in the soil where they were growing and, when differences existed (usually in the upper layers of the soil), the water content of the soil was higher for the female plants than for the male plants.

Conclusions Sex-differential timing of investment in reproduction and differential availability and use of resources from the soil (particularly water) are factors that probably offset the costs of reproduction in the above-ground growth in males and females of *H. peploides*. The results suggest that the patterns of spatial segregation of the sexes observed in *H. peploides* may contribute to maximize each sex's growth and reproduction.

INTRODUCTION

Dioecious plant species, in which different individuals perform different sexual functions, often show sex-specific differences in morphological and physiological traits (Dawson and Ehleringer, 1993; Obeso *et al.*, 1998; Dawson and Geber, 1999; Retuerto *et al.*, 2000; Correia and Díaz Barradas, 2000; Verdú, 2004), in ecological characters, as patterns of defence and herbivory (Krischik and Denno, 1990; Retuerto *et al.*, 2006), survival (Allen and Antos, 1988) or spatial distribution (Iglesias and Bell, 1989), and in patterns of growth and resource allocation (Ågren, 1988; Ramp and Stephenson, 1988; Nicotra, 1999; Leigh *et al.*, 2006). These intersexual differences have commonly been explained as a consequence of the different reproductive functions performed by the sexes. Because females produce seeds and fruits in addition to flowers, many studies have found that females expend proportionally more of their resources on reproduction and less on maintenance and growth compared to males (Lloyd and Webb, 1977; Willson, 1983; Delph, 1999). However, some studies have challenged this generalization, reporting similar or even higher reproductive investment in males due to a greater allocation to floral display (Delph *et al.*, 1993; Leigh *et al.*, 2006) or to pollen in wind-pollinated species such as the herb *Mercurialis annua* (Hesse and Pannell, 2011). Sexual dimorphism in allocation might also arise as result of selection for traits that may allow each gender to meet the specific resource demands associated with reproduction (Cox, 1981; Cipollini and Stiles, 1991; Nicotra *et al.*, 2003; Harris and Pannell, 2008; Sánchez-Vilas and Pannell, 2011).

Sexual differences in resource allocation may be an important determinant of the performance of each sex in different habitats and therefore could help to explain why some populations of dioecious species show habitat-specific sex ratio biases (Freeman *et al.*, 1976; Bierzychudek and Eckhart, 1988). Within-population sex ratio variation, or spatial segregation of the sexes, may occur if

the sexes respond differentially to given environmental conditions. Differences in competitive abilities between the sexes (Cox, 1981; Eppley, 2006), morphological or physiological specialization of the sexes to different habitats (Dawson and Ehleringer, 1993; Dawson and Geber, 1999) or intersexual differences in reproductive biology (Lloyd, 1973; Bierzychudek and Eckhart, 1988) have been suggested as possible causes of spatial segregation. If the reproduction is more costly for females, as it has usually been argued, the relative fitness of females will increase as environmental quality improves and consequently, the sex ratio will become female-biased in high-quality environments and male-biased in stressful or resource poor habitats. In fact, this is the prevailing pattern emerging in the literature on spatial segregation of the sexes (Freeman *et al.*, 1976; Lloyd and Webb, 1977; Freeman *et al.*, 1980; Bierzychudek and Eckhart, 1988; Geber, 1999; Dawson and Geber, 1999).

Most studies on patterns of biomass allocation in dioecious species have examined sexual differences in biomass distribution at a single point in time (Wallace and Rundel, 1979; Bullock, 1984; Hemborg and Karlsson, 1999). However, because sexes may differ in their timing of development (Lloyd and Webb, 1977), static, or single point estimation of patterns of allocation may not reflect real or time-integrated patterns. The few studies that have examined seasonal patterns of biomass distribution have found significant differences between the sexes in the amount of resources allocated, and in the timing of allocation, to reproductive and vegetative structures (Gross and Soulé, 1981; Ågren, 1988; Hemborg and Karlsson, 1999; Ehlers and Thompson, 2004). More research is required for a thorough understanding and realistic modelling of the quantitative relationships between male and female reproductive effort over the whole growing season. We are not aware of any field study considering below-ground structures in the analysis of the patterns of biomass allocation in dimorphic plants. Most of the field studies on allocation have expressed the reproductive effort as the ratio of the dry mass of reproductive tissues to the total dry mass of the above ground tissues (Korpelainen, 1992;

Leigh *et al.*, 2006; Zunzunegui *et al.*, 2006). Although the extent and vertical distribution of the root system has been found to be critical for water uptake and drought tolerance (Moroke *et al.*, 2005; Yu *et al.*, 2007), we know no studies addressing if sexes of dimorphic species differ in vertical root distribution.

Honckenya peploides (L.) Ehrh. (Caryophyllaceae) is a dimorphic species typically found on the upper beach, at the embryo dunes. It presents a breeding system in which male and female flowers are borne on separate plants. Female plants never produce pollen and are constant in their sex expression, whereas "male" plants, all of which produce pollen, may also produce a small number of seeds. We will name to this second sexual morph as male since it attains most of its fitness *via* pollen export. This system, called subdioecy, is close to dioecy in the evolutionary pathway from hermaphroditism to dioecy *via* gynodioecy (see Delph and Wolf, 2005 for a review). At the location of our study, *H. peploides* displays an extreme spatial segregation of the sexes, with monomorphic patches composed exclusively of individuals of either one sex or the other.

The allocation of biomass among below-ground, above-ground and reproductive structures may be especially crucial for dune plants, such as *H. peploides*. Due to the low capacity of sandy soils to buffer against drought, dune plants may experience severe water deficits, especially in spring and summer, when high temperatures lead to high evapotranspiration rates. Under these conditions, sex-specific allocation of biomass to structures specialized in the uptake of water and the scarce nutrients available in the dune systems might result in a differential survival of the sexes in different habitats with the result of a spatial segregation of the sexes. Previous research undertaken in individuals of *H. peploides* growing under greenhouse conditions has found greater reproductive effort and smaller size in females than in males, but not sex-specific differences in the below- : above-ground biomass ratio (Sánchez-Vilas and Retuerto, 2011).

Here, we extend our previous study by comparing the seasonal patterns of soil water availability and biomass allocation in two consecutive years in males and females of *H. peploides* by collecting soil and plant samples in natural populations. Specifically, we aimed to address the following questions: 1) Do males and females of *H. peploides* differ in their seasonal patterns of biomass allocation to below- and above-ground vegetative structures and to reproduction? 2) Do they differ in their vertical distribution of below-ground biomass? 3) Do habitats of male and female plants differ in water availability, as estimated by soil water content?

MATERIAL AND METHODS

Study species

Sea sandwort, *Honckenya peploides*, is a subdioecious perennial plant with a circumpolar distribution (from temperate to arctic zones; anthropochorous in South America). In the Iberian Peninsula, *H. peploides* extends from the Atlantic coast of northern Portugal and northwards and eastwards along the Bay of Biscay (Mar Cantábrico) to France. It is a hemicryptophyte regrowing each spring from long rhizomes that produce compact groups of aerial shoots, forming vegetative patches or mats. These patches are typically found on the upper beach, forming small and unconnected mounds called embryo dunes, which are the youngest dune formations in coastal systems. Flowering takes place from March to June and fruiting occurs from May to the end of August. Flowers are axillary and solitary, and/or in 1- to 6-flowered terminal cymes, strongly honey-scented. Two types of flower can be found in *H. peploides*, as reported for the subspecies *major* by Tsukui and Sugawara (1992). One type (hereinafter “female flower”) has long styles, short petals and non-functional anthers; the other (hereinafter “male flower”) has short styles, long petals and long

stamens that produce pollen grains; this definition of male and female flowers is as per Tsukui and Sugawara (1992), and based on the arguments of Lloyd (1976) and Delph (1990). Male flowers rarely produce seeds, and when they do the number of seeds produced is very small compared to female flowers. Both types of flower have nectaries at the base of the stamens which attract pollinators. Plants reproduce sexually by seed or clonally by adventitious shoots produced by root buds. *H. peploides* is an early colonizer, contributing to stabilization and anchorage of the soil and facilitating the establishment of other species (Houle, 1997; Gagné and Houle, 2001).

Study site

Fieldwork was conducted monthly from April to the end of August of 2006 and 2007 at the Lariño site (42°46'00"N, 9°06'58"W), on the coast of Galicia (NW of Spain). Climatic data (summarized in Figure 1) were obtained from the nearest meteorological station at Corrubedo (42°33'20"N, 9°01'43"W; 25 km away from Lariño) and were provided by the meteorological service of Galicia (www.meteogalicia.es). We studied all the six segregated patches of *H. peploides* plants existing at that site, three composed exclusively of females and the other of male individuals and all of them facing South-West. Patches were separated by at least 10 m from the nearest patch. Individuals from the six monomorphic patches were sexed on the basis of their floral morphology, and the study was conducted during the flowering and fruiting seasons. We have been studying this population during the last five years and we have not observed inconstancy in sex-expression. Moreover, a previous study using amplified fragment length polymorphism (AFLP) as well as isozyme analysis has found considerably high values of genetic variation, revealing several genets within each of these monomorphic patches (Sánchez-Vilas *et al.*, 2010). In Lariño, *H. peploides* grows forming well-delimited patches, in which it is the dominant species; other species including *Cakile maritima*, *Eryngium maritimum* or *Ammophila arenaria* are sometimes present, but at very low

density in the patch.

Soil and plant samples were collected in the early morning in the patches. In each patch, we established transects perpendicular and parallel to the coast line delimiting an x, y coordinate system. We randomly selected x, y coordinates to collect soil and plant samples within each patch. If x, y coordinates corresponded to bare soil we selected the nearest individual to collect the samples. Similarly, we also avoided those points sampled on previous months. In April and May 2006, we collected two samples in each of the six patches. In June, July and August 2006, the size of four of the patches (two male and two female) had increased so much that we decided to increase the number of samples in these patches to four per patch, maintaining two samples in each of the two smallest patches. In 2007, we also collected samples monthly from April to August following the sampling scheme used in June, July and August 2006. Approximately, 2% of the plant cover in each patch was sampled. We used a cylindrical soil core sampler for collecting soil and plant samples at the same point, in order to determine above and below-ground plant mass, and soil moisture. The cylindrical sampler (10 cm diameter, 45 cm length) was driven into the soil and a soil core was carefully removed preserving the plant and soil sample as it existed *in situ*. Samples were taken from the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm depths, by cutting the soil core at these predetermined depths. The 0-10 cm samples included the above-ground biomass. Each sample was put into a sealed plastic bag and this into a zip lock plastic bag. Each sample was identified and carried to the laboratory, where samples were weighed at a ± 0.01 g precision to obtain their total fresh mass. Then, from each sample, we separated the biomass of *H. peploides* and determined its fresh mass. Fresh biomass was subtracted from the total sample mass to obtain soil sample mass. From the 0-10 cm samples we separated above- (shoot) and below-ground (rhizomes and roots) mass of *H. peploides*. We split the above-ground mass into vegetative and reproductive (flower and fruits). In these samples, we also counted the

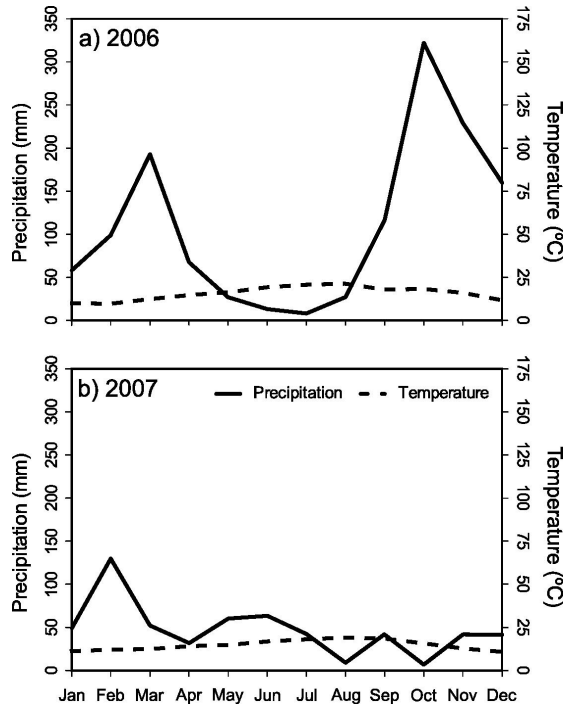


Fig 1. Gaussen's ombrothermic diagrams for 2006 (a) and 2007 (b) obtained from the meteorological station at Corruedo (42°33'20"N, 9°01'43"W). Dashed and solid line represent, respectively, monthly average temperature (°C) and monthly average precipitation in mm in a 2 : 1 (temperature : precipitation) scale. Drought conditions are considered when the precipitation is lower than twice the temperature values.

number of fruits and at the peak of the flowering (April), the number of fresh flowers. Below-ground biomass was washed and all plant material was oven-dried at 55°C for 6 days and weighed at a ± 0.0001 g precision (Mettler AJ100, Switzerland) to calculate dry mass of below-ground structures (including rhizomes and roots), shoots, flowers and fruits. Biomass allocation patterns were assessed by calculating below- : above-ground vegetative ratios and reproductive effort, estimated as the ratio of reproductive to vegetative dry mass (*i.e.*, to above- and below-ground vegetative dry mass). Mean flower and fruit dry mass were estimated from dry flower and fruit mass and the number of flowers and fruits, respectively. After separating *H. peploides* biomass, soil

samples were placed in an oven at 75°C for a minimum of 72 hours for drying. Soil water content at each predetermined depth was obtained by dividing the difference between wet and dry soil masses by the mass of the dry sample to obtain the ratio of the mass of water to the mass of dry soil ($\text{g water} \cdot \text{Kg}^{-1} \text{ dry soil}$). Males and females had similar water content in their below-ground structures ($3.17 \pm 0.147 \text{ g} \cdot \text{g}^{-1}$ and $3.12 \pm 0.179 \text{ g} \cdot \text{g}^{-1}$, respectively; ANOVA test: $F_{1, 237} = 1.17$, $P = 0.280$), calculated as ((below-ground fresh mass - below-ground dry mass) / below-ground dry mass) in a random sample of below-ground structures (roots and rhizomes) of males and females harvested from April to August in 2007 (N = 118 and 121, for males and females respectively).

Statistical analyses

We tested for differences between males and females and among months for total biomass, total above-ground biomass (including vegetative and reproductive biomass), above-ground vegetative biomass, below- : above-ground vegetative biomass ratio and reproductive effort in 2006 and 2007. For each variable, analyses were carried out using linear mixed-effects models using the R “lme” function from the “nlme” package (Pinheiro *et al.*, 2009) in which Sex and Month were fitted as fixed factors and Patch was fitted as random effect. In these analyses, tests of significance were carried out using an *F*-test based on marginal sums of squares using the function “anova” for each model object (Pinheiro and Bates, 2000). Total biomass, flower biomass and below- : above-ground vegetative biomass ratio were square-root transformed, total below-ground biomass for 2007 was \log_{10} -transformed to achieve normality of standardized residuals and homogeneity of variance.

To analyse the below-ground biomass and the water content at different soil depths, we performed linear mixed-effect models using the R “lmer” function from “lme4” package (Bates and Maechler, 2010). This function

handles models with more complex random structures than “lme”, allowing the nesting of random effects. In these analyses we fitted Sex, Month and Depth as fixed factors and Patch and Core (nested within Patch) as random effects. Significance of the fixed effects was assessed by means of likelihood ratio tests by calling the function “anova” to compare models with and without the factor being tested that are fitted using maximum likelihood estimates (Pinheiro and Bates, 2000; Crawley, 2007). Below-ground biomass for 2006 and 2007, and water content for 2007 were \log_{10} -transformed.

All statistical analyses were carried out in R version 2.8.1 (R Development Core Team, 2008).

RESULTS

Biomass and reproductive traits

The reproductive effort (proportion of biomass allocated to reproductive structures) of males and females changed differently over time (Table 1, Fig. 2, Sex \times Month interaction). Males allocated proportionally more to reproductive structures at earlier stages than did females; however as season progresses, females were allocating more biomass to reproduction than males.

We did not find significant differences in total above-ground biomass, above-ground vegetative biomass, total biomass and below- : above-ground vegetative biomass ratio between males and females (Table 1). However, these variables changed significantly over time (except total biomass in 2007; Table 1). In 2006, total above-ground biomass, above-ground vegetative biomass and total biomass showed an increase from April to May, and then decreased again until reaching similar values to those found in April (Figs. 3a and 3b for total above-ground and total biomass, similar patterns were followed by above-ground vegetative biomass (not shown in figure)). In 2007, total above-ground and above-ground vegetative biomass increased significantly over time

(Fig. 3d for total above-ground biomass), whilst no changes were observed in total biomass (Fig. 3e). Below- : above-ground vegetative biomass ratio decreased with time in both years (Figs. 3c and 3f).

In 2006, we found differences in below-ground biomass of males and females that varied with depth (Sex \times Depth interaction: $\chi^2 = 5.48$, $P = 0.006$). Both sexes showed similar below-ground biomass on the first layer of soil sampled (0-10 cm), but showed differences in the rest of layers of soil, with females having greater values than males, especially in the deepest soil layer (30-40 cm) (Fig. 4). Overall, and regardless of the Sex and Depth, the below-ground biomass declined with time from May to August ($\chi^2 = 40.3$, $P < 0.0001$; Fig. 4). In 2007, we also found differences in below-ground biomass of males and females, which varied with Depth and time (Sex \times Depth \times Month interaction: $\chi^2 = 25.8$, $P = 0.012$). Although this three-way interaction is somewhat complex and differences depended on Depth, we can observe that females usually had greater values than males, especially in the months of July and August (Fig. 4).

Soil water content

Differences between soil water content in the habitat of males and females depended on Depth in both years (Sex \times Depth interaction: $\chi^2 = 13.6$, $P = 0.003$ for 2006 and $\chi^2 = 27.08$, $P < 0.001$ for 2007). In 2006, the first layer of the soil (0-10 cm) has greater soil water content than the second layer (10-20 cm), especially for females, and similar values to the rest of the other layers (Fig. 5). In 2007, water content increases with increasing depth. In both years, 2006 and 2007, we found that females had greater water content near the soil surface than males, especially in the 0-10 cm. However, these differences disappeared in the deepest soil layers (Fig. 5).

In addition, water soil content decreased with time, although this decrease differed among the different soil layers (Depth \times Month interaction: $\chi^2 = 36.8$,

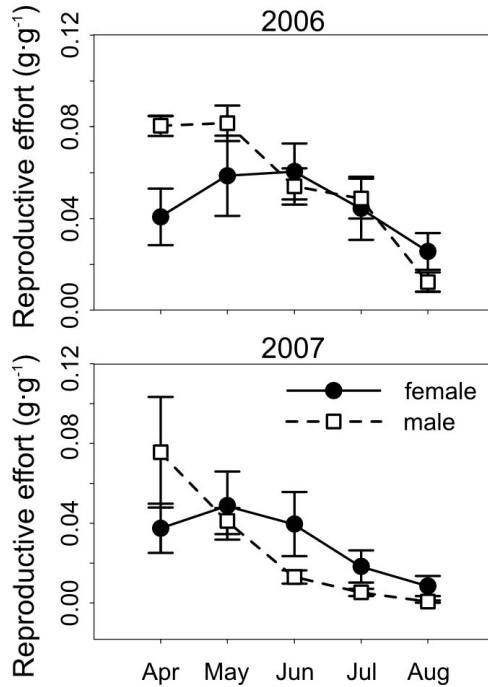


Fig. 2. Seasonal variation in reproductive effort (reproductive / vegetative biomass) of males and females of *Honckenya peploides* during 2006 and 2007. Values represent means ($n = 10$, except $n = 6$ for April and May 2006) \pm S.E.

$P < 0.001$ for 2006 and $\chi^2 = 28.5$, $P < 0.001$ for 2007). In particular, the water content in the surface decreased less than in the deepest layers of the soil in 2006, whilst the opposite pattern was true for 2007; Fig. 5). The temporal patterns of water soil content did not vary for males and females (Sex \times Month interaction: $\chi^2 = 3.12$, $P = 0.538$ for 2006 and $\chi^2 = 5.82$, $P = 0.589$ for 2007).

Table 1. Results of the mixed effect models for the reproductive effort, total above-ground biomass, above-ground vegetative biomass, total biomass and below:-above-ground vegetative biomass ratio. Patch was included in the analysis as random variable (not shown) and Sex, Month and their interaction were treated as fixed factors. Numbers in parenthesis are the degrees of freedom of numerator and denominator used to test the fixed factors. *P*-values < 0.05 are shown in bold type.

	2006						2007					
	Sex (1,4)		Month (4,70)		Sex × Month (4,70)		Sex (1,4)		Month (4,86)		Sex × month (4,86)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Reproductive effort	4.42	0.103	2.41	0.058	2.10	0.090	2.54	0.186	2.37	0.058	2.58	0.043
Total above-ground biomass	0.133	0.734	5.57	0.001	1.28	0.284	0.820	0.416	3.61	0.009	1.12	0.351
Above-ground vegetative biomass	0.0387	0.854	4.40	0.003	1.13	0.351	1.15	0.343	4.84	0.001	1.31	0.273
Total biomass	0.585	0.487	13.3	< 0.001	1.19	0.322	0.898	0.397	0.670	0.615	0.810	0.522
Below:-above-ground vegetative biomass	2.47	0.191	6.35	< 0.001	1.31	0.276	0.510	0.515	3.79	0.007	0.184	0.946

DISCUSSION

Sex differences in reproductive allocation

Our study revealed different patterns of biomass allocation to reproduction in males and females of *H. peploides*. Males decreased their relative allocation to reproduction over time due to most of the flowering taking place until May. Females maintained roughly constant their allocation to reproduction from April to June, presumably as a consequence of maturing fruits after

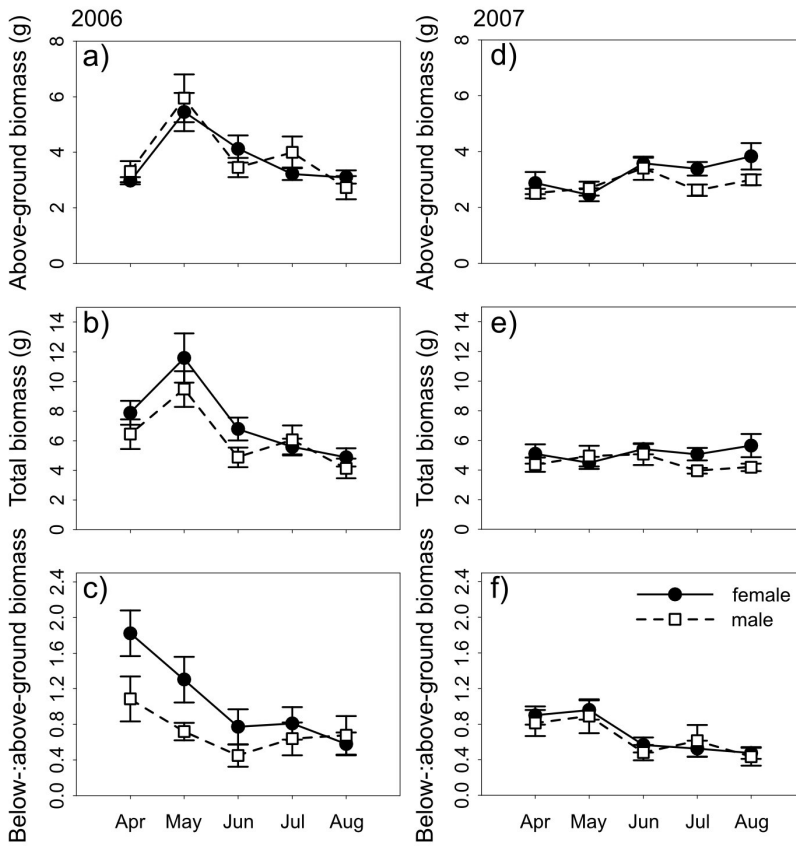


Fig. 3. Mean monthly values (± S.E.) of total above-ground and total biomass and below- : above-ground vegetative biomass ratio in males and females of *H. peploides* from April to August in 2006 (a-c) and 2007 (d-f).

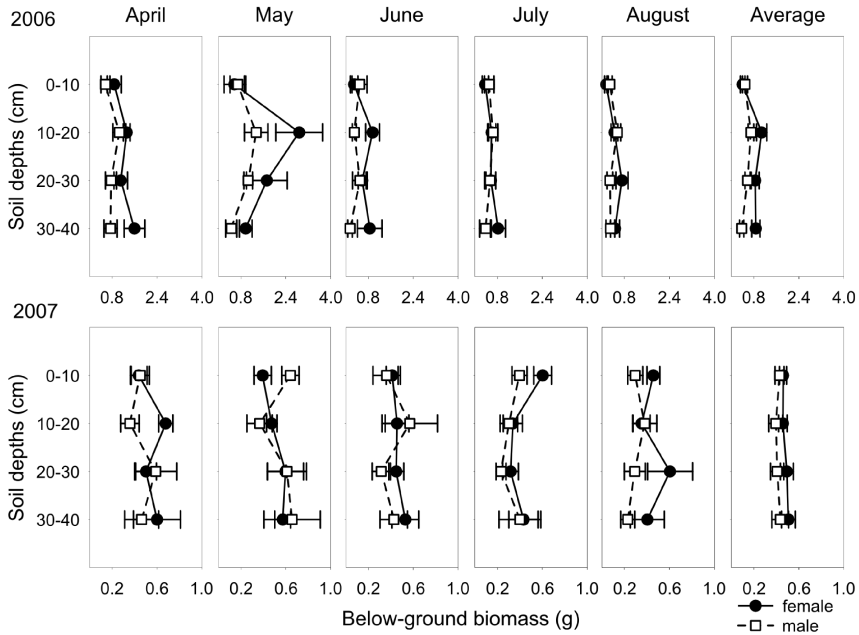


Fig. 4. Vertical distribution of below-ground biomass (g) of males and females of *H. peplodes* in the soil profile for April, May, June, July, August and the average for all months in 2006 and 2007. Values represent means ($n = 10$, except $n = 6$ for April and May 2006) \pm S.E. Note: range of below-ground biomass (X-axis) in 2006 is four times greater than in 2007.

flowering has ceased. However, they also showed a decrease in the allocation to reproduction with time, but less pronounced than in the case of males, which corresponds to the dispersion of seeds that are lost from the mother plant. The result is that males tended to have greater reproductive effort than females early in the season (April and May) due to a greater investment in flowers, but sexual differences became diminished or even reversed as the season progressed. The pattern of allocation to reproduction in females is somewhat surprising, because a much greater increase in May-June due to fruit production could be expected. This low fruit production in females might be attributable to pollen limitation due to the spatial segregation of males and

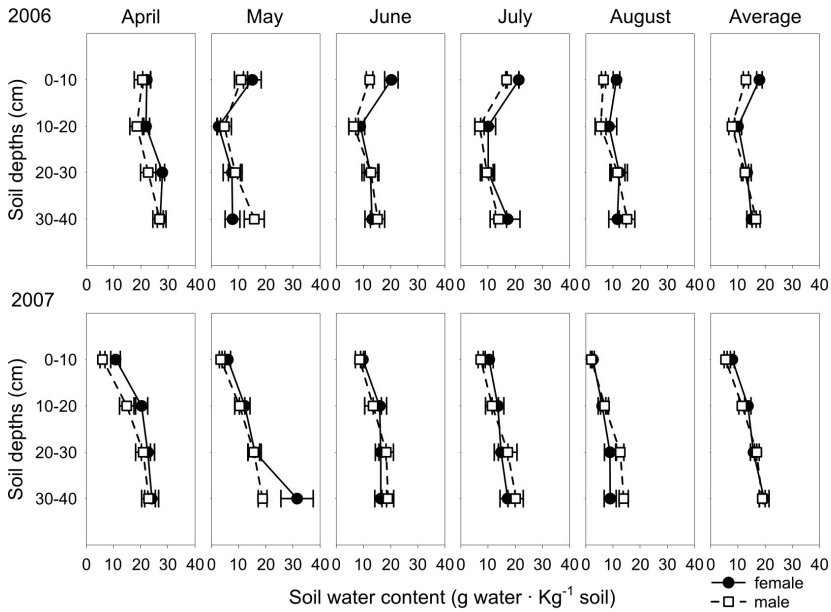


Fig. 5. Soil water content ($\text{g water} \cdot \text{Kg}^{-1} \text{ soil}$) in male and female habitats of *Honckenya peploides* from April to August 2006 and 2007. Values represent means ($n = 10$, except $n = 6$ for April and May 2006) \pm S.E. The average values for all months are also shown.

female in different patches. Different patterns in the biomass allocation to reproduction between males and females have been previously identified in dioecious plants, mainly woody species, with males allocating more biomass to reproduction than females at flowering and females more to reproduction than males at fruiting (e.g. Allen and Antos, 1988; Delph *et al.*, 1993; Nicotra, 1999; Obeso, 2002; Leigh and Nicotra, 2003). Since the energetic requirements for producing fruits are generally greater than for flower production, and the maturation of fruits usually extends for a considerable period after flowering, females must continue to expend resources longer than do males as observed in our study. Consequently, females of dioecious plants typically have been found to have a higher investment in reproduction than males over an entire growing season (Lloyd and Webb, 1977; Gross and

Soulé, 1981; Willson, 1986; Popp and Reinartz, 1988; Leigh *et al.*, 2006). For these reasons, many studies have found higher vegetative growth in males of dioecious plants at the end of the growing season, which is consistent with the existence of a trade-off investment between reproductive and vegetative biomass (Herrera, 1988; Vasiliauskas and Aarsen, 1992; Gibson and Menges, 1994; Watson, 1995; Díaz Barradas and Correia, 1999; Hemborg and Karlsson, 1999). Such trend has also been found in *H. peploides* growing under greenhouse conditions, where females had over four times greater reproductive effort than males, which in turn had a greater size than females (Sánchez-Vilas and Retuerto, 2011). However, here we have not found the expected intersexual differences in the above-ground vegetative growth: males and females did not differ in their temporal patterns of growth and female growth was not reduced relative to males towards the end of the growing season.

The timing of resource investment in reproduction may be as, or more, important than the amount invested in shaping sexual differences in growth (Gross and Soulé, 1981; Eckhart and Seger, 1999; Case and Ashman, 2005; Sánchez-Vilas and Pannell, 2011). The greater allocation of resources to reproduction in males early in the season may divert those resources away from investment in photosynthetic machinery that might otherwise contribute to an increased growth rate (Obeso, 2002). This may partly explain why comparatively greater reproductive effort in females towards the end of the season does not negatively impact their growth in comparison to males. In addition, the lack of sex-specific differences in growth may be due to similar cost of reproduction in both sexes, as a consequence of the low fruit production of females in the two years of the study.

Sex differences in below-ground allocation

Despite of the lack of differences in above-ground vegetative biomass

between males and females of *H. peploides*, we did observe sexual differences in the patterns of allocation to below-ground structures. On the one hand, females had greater below-ground biomass, but only at certain soil depths. The greater below-ground biomass in females than in males found in our study could be due to either an increased amount of fine roots or/and to an increased rhizome growth; the particular contribution of each of these two components to the below-ground biomass is unknown in our study. A greater amount of fine roots could be expected if females had a greater demand of water and nutrients due to fruit production and maturation. However, since male and females showed similar reproductive effort, especially in 2006, other factors may be shaping this difference. Due to the perennial habit of this species, the sex-differential investment in below-ground biomass may also be related, as pointed out above, to sex-specific differences in the patterns of allocation to storage (*i.e.*, rhizomes). Sex-specific differences on biomass allocation to storage organs have been previously found in other dioecious species, for example, in *Borderea pyrenaica* (Garcia and Antor, 1995) and *Corema album* (Alvarez-Cansino *et al.*, 2010). On the other hand, regardless of the Sex, we observed temporal variability in below-ground biomass: the below-ground biomass in the different layers of soil changed differently throughout the season. Optimal allocation theory predicts that plants will maximize growth under a given set of resource conditions by allocating biomass to the organ responsible for acquiring the most limiting resource (Bloom *et al.*, 1985; Bazzaz, 1997). Plants should thus increase allocation to shoots in conditions of carbon stress (e.g., resulting from shading) and to roots in conditions of nutrients or water stress (Bloom *et al.*, 1985). We might also expect an increase in allocation to the storage organs (rhizomes) as the season progressed. However, we found a decrease in below- : above-ground vegetative biomass ratio as the season progressed and water stress became greater (see below). Different precipitation patterns in 2006 and 2007 indicate that water stress may be not so strong in the summer of 2007 (see figure 1). In

2007, spring was quite dry in comparison to the previous year, but in summer the amount of precipitation was higher than in the same period for 2006. The result is that above-ground biomass increased with time in 2007, which may have shifted the below- : above-ground vegetative biomass ratio towards lower values as the season progressed in this year. Nevertheless, the decrease in below- : above-ground vegetative biomass ratio is somewhat surprising, especially for 2006, where summer drought is intense, considering that a larger proportion of assimilates allocated to root production may be particularly significant in maintaining an adequate water balance (Aronson *et al.*, 1992; Retuerto and Woodward, 1993; Fitter and Hay, 2002). Although we know no field studies examining seasonal changes in below-ground allocation in dioecious plants, some authors have reported results consistent with our findings. In *Silene latifolia* the proportion of biomass allocated to roots decreased in time with allocation to reproduction (Gehring, 1993). Escarré *et al.* (1990) reported decreased below- : above-ground vegetative biomass ratios in females of *Rumex acetosella* during flowering and fruiting. Similarly, Cibils *et al.* (2005) found that females of *Atriplex canescens* produced less roots than males when soil moisture was deficient. These authors suggested that females were constrained by their relative inefficient use of water and were unable to produce sufficient photosynthates to sustain a greater allocation to the root system. Our results highlight the possibility that the ability to alter timing and placement of root proliferation, as indicated by temporal variability and differences with Depth in below-ground biomass, may be more important for plant success than changes in the partitioning of biomass between below- and above-ground structures (Reynolds and D'Antonio, 1996).

Sex differences in habitat

Sexes of many dioecious plant species are spatially segregated along a gradient of habitat quality, with females being more common under less stressful conditions, i.e., in sites that are moister, less exposed, less saline,

and/or with higher nutrient concentrations (Bierzychudek and Eckhart, 1988). In line with this, in our study the first layers of the soil where females were growing showed higher water content than those of males. However, the differences were less clear at other depths, which makes it difficult to relate solely this factor to the presence/absence of the sexes as previously have been done for other species (Dawson and Bliss, 1989; Dawson and Ehleringer, 1993).

Soil water content decreased towards the end of the season, in both male and female habitats. Climatic conditions characterised by increasing temperatures and radiation and lower precipitation as summer progresses, may explain this decrease in the content of water in the soil. However, and contrary to what we might expect, this decrease was similar in female and in male habitats (or even tended to be more pronounced in female than in male habitats). Towards the end of the season, females are making greater investment in reproduction than males, so reduced water availability may critically affect their growth. However, no differences between males and females were found in above-ground growth. A plausible explanation for this unexpected result is a differential use of this limiting resource by males and females. Of course, our study did not measure directly the plant water status, and therefore we lack empirical evidence for such explanation. Nonetheless, in support of this hypothesis, we found greater below-ground biomass in females, which presumably contributes to increase the uptake of water more in females than in males when this becomes scarce. This greater use of water by females would also explain a similar water content of female and male habitats in the deepest layers of the soil despite of females having greater water content in the upper layers. Greater below-ground biomass, as pointed above, may be also related to greater allocation to rhizomes, which are mainly storage organs. A high capacity for water storage by rhizomes has been found in species of arid environments, such as *Leymus chinensis*, playing an important role in regulating plant growth (Wang *et al.*, 2008). In this sense, a

greater water storage capacity of females would be in accordance with previous evidences that found different strategies to cope with water stress in the sexes of *H. peploides*, with females displaying mechanisms to maintain more favourable tissue water content than males (Sánchez-Vilas and Retuerto, 2009). These results would indicate some degree of sexual specialization in resource acquisition and utilization, suggesting that males and females may benefit if they occupy different niches within the environment (i.e. niche partitioning; Freeman *et al.*, 1976; Onyekwelu and Harper, 1979; Cox, 1981; Vitale *et al.*, 1987; Bierzychudek and Eckhart, 1988; Freeman *et al.*, 1997; Dawson and Geber, 1999; Sánchez-Vilas and Pannell, 2010).

Finally, our seasonal analysis of soil water content suggests that timing of investment in reproduction in males occurs when moisture in the soil is high, as pointed above. Males of *H. peploides* tend to allocate proportionally more biomass to reproduction than females early in the season. Since water is less limiting at this time than later in the season, males wouldn't need to invest as much resources as females in root production, resulting in greater below-ground biomass in females than in males.

CONCLUSION

Males and females of *H. peploides* had different profiles of water content in the soil where they were growing. Overall, when differences existed (usually in the upper layers of the soil), females had higher soil water content than males. Similarly, the allocation of biomass to below-ground structures also varied in Depth differently for males and females, with females usually having greater below-ground biomass than males. Although males and females differed in patterns of reproductive effort, no differences were found in above-ground growth. Sex differential timing of investment in reproduction —males invest more biomass to reproduction early in the season, when water content is high— and differential availability and use of resources from the soil

(particularly water) are all factors that probably offset the costs of reproduction in above-ground growth in males and females of *H. peploides*. Therefore, our results found evidences to support that the patterns of spatial segregation of the sexes observed in *H. peploides* may contribute to maximize each sex's growth and reproduction, although further studies are needed to fully understand the mechanisms that create such patterns.

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Discusión y Conclusiones generales

DISCUSIÓN GENERAL

A partir de la integración de los resultados obtenidos en los diferentes trabajos, podemos confirmar que en las especies de plantas de los ecosistemas dunares costeros existe una marcada variabilidad, a un nivel ecofisiológico, para diferentes rasgos relacionados con el rendimiento. Esa variabilidad, además, se observó en todos los diferentes tipos de rasgos funcionales estudiados. La variabilidad global en las plantas de estos ambientes fue mayor que la detectada para los factores estudiados en cada uno de los capítulos por separado, pues donde para los factores vistos en un capítulo algunas especies parecían mostrar ciertas similitudes, en otro, donde se estudiaban unos rasgos distintos, mostraban marcadas diferencias. Esta gran divergencia, observada en los rasgos estudiados, contradice lo pronosticado por la teoría del filtrado ambiental (Keddy, 1992; Díaz, 1998; Mouillot, 2007), que sí se cumple en otros ambientes, caracterizados también por fuertes presiones abióticas. Esa divergencia sería más afín con lo que predice la teoría del límite de similitud (MacArthur, 1967; Stubbs, 2004; Mouillot, 2007), que es la empleada habitualmente para explicar los procesos divergentes en otros ambientes y que se fundamenta en interacciones competitivas. Por otro lado, también es muy probable que se deba a procesos de perturbación, como predice la teoría de los filtros de perturbación (Grime, 2006), en concordancia con la gran influencia de los factores abióticos y la variabilidad e impredecibilidad característicos de estos ambientes.

Si bien la gran mayoría de los rasgos estudiados mostraron gran variabilidad y divergencia, para otros, como la capacidad de recuperación tras la exposición a una radiación saturante, las diferentes especies mostraron una capacidad de recuperación muy parecida, alcanzando valores muy similares y en un breve espacio de tiempo. Esto demuestra que, si bien la forma de procesar la radiación fue variable, su capacidad de recuperación sí resultó ser

mucho más conservada.

A consecuencia de la variabilidad encontrada tanto para las diferentes configuraciones de rasgos como para los rasgos individuales, las especies presentan estrategias claramente contrastadas. Esto impide que si una especie desaparece pueda ser reemplazada por otra equivalente, de similares características y que desempeñe las mismas funciones. Si bien la ausencia, temporal o permanente, de alguna de estas especies implicaría la pérdida de una combinación de rasgos única, a nivel de comunidad es posible que pueda ser compensada por el conjunto de las demás especies.

Otra característica que observamos es que no se encontró ninguna especie con una combinación de rasgos que tenga una ventaja clara sobre los demás. Esto podría explicarse debido a las características de las condiciones ambientales de estos sistemas, altamente cambiantes e impredecibles, que hacen que no existan unas condiciones concretas y estables a las que las especies puedan adaptarse. Las configuraciones de rasgos particulares que adquirió cada especie, hace que cada una tenga su óptimo bajo diferentes condiciones ambientales. Esto explicaría, al menos en parte, el alto grado de divergencia de rasgos funcionales y estrategias adquiridas por estas especies en este tipo de ambientes, caracterizados por severas presiones ambientales.

A partir de los resultados obtenidos en los diferentes capítulos, pudimos observar que la gran variabilidad mostrada por estas plantas resultó ser altamente dependiente de las especies seleccionadas, el momento y el lugar donde se toman las medidas, así como de las características propias de cada rasgo funcional estudiado. Con esto, podemos afirmar que no existe ninguna técnica que podamos emplear (o rasgo que podamos cuantificar), a partir de la cual podamos determinar de forma global el estado y la complejidad contenidas en las especies de los ambientes dunares costeros. Para poder lograr una buena aproximación, recomendaríamos tomar las muestras en diferentes momentos, en diferentes posiciones, seleccionar diferentes especies

y cuantificar diferentes tipos de rasgos funcionales. De no satisfacerse estas condiciones nos estaríamos arriesgando a subestimar la variabilidad y complejidad contenidas en estos ambientes, desde un punto de vista ecofisiológico.

CONCLUSIONES:

Capítulo 1

Las especies que coexisten en el frente dunar se diferencian ampliamente en varios rasgos funcionales claves para la planta, relacionados con el uso de recursos esenciales tales como el agua, el nitrógeno y la radiación.

Los resultados apoyan la idea de que las especies pueden coexistir más exitosamente si reducen la competencia, diferenciándose en sus estrategias en el uso de recursos. Se detectó una segregación temporal de nichos en la explotación de recursos por las diferentes especies, lo que facilita su coexistencia.

Contrariamente a la esperada convergencia de rasgos, debido a la convergencia funcional, nuestros resultados muestran que el proceso de límite de similitud puede resultar más determinante en la estructuración de estas comunidades. Sin embargo, estos resultados también son consistentes con la idea de una comunidad en un estado de falta de equilibrio, en el que el estado de equilibrio se ve impedido por una amplia gama de factores.

Aunque las diferencias de los rasgos de las especies afectan a su vulnerabilidad a cambios ambientales, la contribución de procesos de diferente naturaleza (constricciones ambientales, perturbaciones, etc.) a la

estructuración de las comunidades hace difícil el predecir como se verá afectada en el futuro la coexistencia de las especies.

La marcada segregación, en cuanto a los espacios funcionales de las especies que coexisten, sugiere una amplia diversidad funcional y complejidad en los ambientes costeros.

Posibles cambios en estos ambientes, amenazados por factores como la expansión urbana, las invasiones biológicas o un incremento en el nivel del mar, pueden terminar con procesos únicos en estos sistemas, pudiendo tener consecuencias importantes en el funcionamiento de estos ecosistemas y en los servicios que proporcionan.

Capítulo 2

Contrariamente a lo ocurrido en otros ambientes de fuerte dureza ambiental, las especies dunares costeras muestran una divergencia significativa en sus rasgos funcionales, tales como el contenido en agua y en pigmentos relacionados con la captación y procesado de la radiación, que les permite adquirir diferentes estrategias, haciendo compatible su coexistencia en el frente dunar.

Nuestros resultados apoyan la hipótesis de que no hay una única configuración óptima de rasgos para estos ambientes sino que, debido a la complejidad de las interacciones y de las soluciones de compromiso entre múltiples rasgos, pueden producirse diversos diseños funcionales alternativos.

Nuestros resultados indican que los procesos que promueven la divergencia, como el caso del límite de similitud, o los procesos de perturbación, tienen importantes implicaciones en la estructuración de las

comunidades dunares.

Nuestros resultados apoyan la idea de la tendencia hacia un equilibrio dinámico, donde las condiciones de equilibrio son modificadas por múltiples soluciones de compromiso, relacionadas con las interacciones entre diferentes factores de estrés y perturbaciones, además de la gran impredecibilidad característica del frente dunar.

El alto nivel de variación, encontrado en todos los rasgos funcionales, relacionados con el contenido hídrico y pigmentos en las hojas y la eficiencia fotoquímica, sugiere un alto grado de diversidad funcional en los procesos del ecosistema. Nuestro trabajo pone de manifiesto la necesidad de conservar la composición de especies para preservar los procesos funcionales que pueden ser específicos de estos ambientes.

Capítulo 3

Nuestros resultados muestran que algunos gradientes ambientales no son percibidos como tales por las especies, o son percibidos como no direccionales. Modificaciones en las condiciones del hábitat, así como la selección de microhábitats por las especies y las complejas interacciones entre factores, pueden alterar el modo en que las especies perciben la direccionalidad de los gradientes abióticos.

Las diferencias observadas a lo largo de gradientes espaciales en rasgos funcionales y estructurales relacionadas con el uso de recursos tales como la luz, el agua y los nutrientes, además de algunas características del suelo, sugieren que las diferentes especies desarrollaron distintas estrategias para sobrevivir a las severas condiciones limitantes de las dunas costeras.

Diseños alternativos de similar eficacia pueden coexistir simultáneamente en un mismo ambiente. La baja correspondencia entre los patrones de variación de rasgos foliares y variables del suelo, nos permiten concluir que las especies raramente responden exclusivamente a la acción de un único factor ambiental.

Los procesos que conducen a la convergencia o a la divergencia en los rasgos funcionales de las especies tienen una importancia similar a lo largo del gradiente dunar.

Capítulo 4

Las especies que coexisten en un área homogénea, y de alta dureza ambiental, en los ambientes dunares, muestran una clara divergencia en varios aspectos de sus procesos fotoquímicos que indican la coexistencia de diferentes estrategias relacionadas con la eficiencia en el procesado de radiación a través de los fotosistemas.

Las diferencias encontradas entre las especies concuerdan con las diferencias observadas en sus morfologías foliares y formas de crecimiento. Sin embargo contradice lo esperado por la teoría del filtrado ambiental para ambientes hostiles, de una reducción del rango de posibles diseños funcionales.

Las diferencias estacionales en los rasgos funcionales de las especies sugieren que su comportamiento relativo, en cuanto a sus eficiencias fotoquímicas, varían con el tiempo. Cuando las condiciones ambientales varían a lo largo del tiempo, y los rendimientos relativos de las especies también cambian, las variaciones ambientales pueden permitir la coexistencia de especies donde, bajo condiciones estables, una excluiría a las demás.

Los cambios a lo largo de un gradiente dunar, para algunos rasgos funcionales de las hojas de que cuantifican la eficiencia fotoquímica del fotosistema II y la disipación térmica, no fueron consistentes con los cambios direccionales asumidos en la severidad de los factores de estrés.

La complejidad de los factores ambientales que influyen en el rendimiento, variable en el espacio y en el tiempo, puede determinar patrones de variación impredecibles, a lo largo del gradiente dunar, en rasgos foliares relacionados con el funcionamiento de varios aspectos de la fotoquímica del fotosistema II.

A pesar de las importantes variaciones observadas en rasgos funcionales, relacionados con la capacidad fotoquímica y mecanismos de disipación del exceso de energía absorbida, encontramos que las plantas muestran patrones de recuperación en condiciones de oscuridad muy similares entre sí. Estos patrones resultaron ser independientes de las especies, la estación o el conjunto de condiciones ambientales estudiado, ya sean diferentes especies coexistiendo en un área homogénea o una única especie a lo largo de un gradiente ambiental. Esto sugiere que las presiones evolutivas que están actuando pueden estar manteniendo mecanismos de recuperación muy similares.

Capítulo 5

Los machos y las hembras de *Honckenya peploides* muestran diferentes patrones de contenido en agua del suelo en los lugares donde crecen.

Cuando se encuentran diferencias (normalmente en las capas superiores del suelo) las hembras tienen mayor contenido en agua que los machos. Algo similar ocurre también con la asignación de biomasa a las estructuras

subterráneas para las que, cuando se detectan diferencias, suele ser también mayor en hembras.

A pesar de que machos y hembras difieren en cuanto al esfuerzo reproductivo, no muestran diferencias en cuanto a su biomasa aérea. Aunque sí difieren en el momento de su máxima inversión en reproducción - siendo los machos quienes invierten en biomasa reproductiva más tempranamente, cuando la disponibilidad de agua es mayor. La diferente disponibilidad y uso de los recursos del suelo (principalmente el agua) son factores que probablemente compensen los costes de reproducción en biomasa aérea en machos y hembras de *H. peploides*.

Nuestros resultados aportan pruebas que sustentan que los patrones de segregación espacial entre sexos, observados en *H. peploides*, pueden contribuir a maximizar el crecimiento y la reproducción de cada sexo, aunque es necesario un mayor estudio para comprender completamente los mecanismos que crean estos patrones.

General

Por último, de forma global, también podemos concluir que:

La gran variabilidad y diversidad observadas indican que, a nivel ecofisiológico, existe un mayor grado de divergencia del esperado para ambientes de marcada dureza ambiental. Podemos afirmar por tanto que en los ecosistemas dunares costeros ocurren simultáneamente importantes procesos tanto de convergencia como de divergencia.

Las especies de los ecosistemas dunares se encuentran en constante cambio, intentando adaptarse a las nuevas condiciones de equilibrio, debido a

la naturaleza altamente cambiante de estos ambientes, derivada de la alta variabilidad e impredecibilidad de los factores de estrés y perturbaciones.

Para una buena aproximación a esta complejidad sería necesario aplicar diferentes técnicas que recogen distintos tipos de información, medir en diversas especies, en diferentes momentos a lo largo del ciclo de crecimiento y en distintas posiciones a lo largo del rango de distribución de las especies.

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Resumen

Los ambientes dunares costeros se caracterizan por una elevada dureza ambiental, causada por la severidad de multitud de factores de estrés, principalmente de origen abiótico, como la salinidad, la movilidad del sustrato, la baja capacidad de retención de agua y nutrientes, el efecto del viento, las altas temperaturas y radiación, etc. Las especies de plantas que habitan estos ambientes, desarrollan una serie de adaptaciones que les permiten tolerar esos factores de estrés. Algunos ejemplos de esas adaptaciones son la adquisición de pubescencia y/o una cutícula engrosada, coloraciones claras de las superficies, adquisición de estructuras de almacenamiento de reservas, gran profundidad de enraizamiento, plegado foliar, etc. Generalmente, los factores de estrés varían en intensidad a lo largo de un gradiente costa-interior, adquiriendo normalmente una mayor severidad cuanto más cerca de la costa nos encontremos. Este hecho, junto a las diferentes capacidades de las plantas para desarrollar las diversas adaptaciones, son los responsables de la zonación de la vegetación típica de estos ambientes, que tiende a formar cinturones paralelos a la línea de costa. A su vez, por un lado, las fuertes presiones abióticas que afectan a estos sistemas podrían hacernos pensar en el predominio de procesos de convergencia, por lo que cabría esperar que las diferentes especies mostrasen una gran similitud en cuanto a rasgos funcionales, tal y como predice la teoría de filtros ambientales. Por contra, la alternativa más frecuente a esta teoría es la del límite de similitud, que predice una divergencia en cuanto a los rasgos funcionales adquiridos por las diferentes especies, y que es justificado por procesos de competencia que impiden que las especies tengan rasgos muy similares. A pesar de la fuerte dureza ambiental característica de estos ambientes, al menos a nivel de morfología foliar y formas de crecimiento, no se observa el grado de convergencia esperado por la teoría del filtrado ambiental. El objetivo de este trabajo es descubrir lo que ocurre a nivel ecofisiológico para factores relacionados con el rendimiento, aspectos que se encuentran mucho menos estudiados. Para ello pretendemos cuantificar y comparar, las variabilidades, a

nivel espacial, temporal y de especies, para variables del rendimiento en diferentes especies. Se trata también de identificar y comparar las diferentes estrategias que puedan adquirir. Para ello, empleamos varias de las principales técnicas ecofisiológicas que nos permiten determinar diferentes rasgos funcionales, claves para el desarrollo y supervivencia de las plantas.

Para poder cumplir estos objetivos se seleccionaron seis especies de herbáceas perennes, comunes en el frente dunar, que mostraron una contrastada morfología foliar y forma de crecimiento: *Eryngium maritimum* L. (Umbelliferae) es una planta hemicriptófita, de hojas plegadas, con espinas, y cutícula engrosada, de coloración clara. *Euphorbia paralias* L. (Euphorbiaceae) es una planta caméfito de hojas pequeñas y claras situadas hacia el ápice de los tallos, que pueden ser vegetativos o reproductivos. Además de C₃ pueden presentar facultativamente metabolismos tipo C₄ y/o CAM. *Calystegia soldanella* (L.) R. Br. (Convolvulaceae) es una planta hemicriptófita, vivaz, de pequeño tamaño, con hojas arriñonadas y algo carnosas. Además de metabolismo tipo C₃ también puede adquirir facultativamente el C₄. *Pancratium maritimum* L. (Amaryllidaceae) es una planta criptófita bulbosa de hojas carnosas suculentas. En cuanto a su morfología son alargadas, estrechas y gruesas, y su color verde-glaucoso. *Matthiola sinuata* (L.) R. Br. (Brassicaceae) es una planta hemicriptófita, con hojas plegadas, pubescentes y de coloración clara. *Honckenya peploides* (L.) Ehrh. (Caryophyllaceae) es una especie hemicriptófita dioica, típica de dunas embrionarias, de hojas pequeñas y suculentas. Crece formando manchas, que frecuentemente son unisexuales (no clonales).

Las localidades de estudio fueron seleccionadas en diferentes sistemas dunares de la costa de Galicia (NO de España). Los experimentos de coexistencia de especies se realizaron en el frente dunar del sistema dunar de Lariño (42°46'00"N, 9°06'58"W), mientras que para los experimentos de gradientes espaciales se seleccionaron los sistemas dunares de Valdoviño (43°36'44"N, 8°10'58"O) y Nerga (42°15'50"N, 8°51'10"O).

En el **capítulo 1**, estudiamos las respuestas de diferentes especies que coexisten en un área homogénea y reducida, a dos de los principales recursos para las plantas (el agua y el nitrógeno). Estudios sobre la estructuración de las comunidades sugieren que los factores de estrés pueden limitar profundamente el rango de respuestas fisiológicas de las plantas en ambientes extremos. En los ambientes dunares costeros se espera una convergencia en los rasgos funcionales, debido a procesos de filtrado ambiental, causados por la severidad de los factores abióticos. Sin embargo el amplio rango de rasgos morfológicos y fenológicos mostrados por las especies de ambientes dunares costeros sugieren que el límite de similitud y/o procesos de perturbación causan una considerable divergencia de los rasgos. Por todo esto, nosotros predecimos que la supervivencia de las plantas en este tipo de ambientes puede ser alcanzada mediante diferentes estrategias. Para ello seleccionamos cinco especies herbáceas perennes, de contrastados rasgos morfológicos y funcionales, que coexisten en un área homogénea y de reducidas dimensiones (220 m²) del frente dunar costero de Lariño (Galicia, NO de España). Sobre ejemplares de estas especies, se midieron diferentes rasgos funcionales de las hojas, así como diferentes parámetros fisicoquímicos del suelo donde crecían. El proceso se repitió en tres ocasiones a lo largo del año 2008, concretamente en los meses de abril, junio y noviembre. Los principales rasgos medidos sobre las plantas fueron la eficiencia integrada en el uso del agua (estimada a partir de la discriminación isotópica del carbono: $\Delta^{13}\text{C}$) y las estrategias para la adquisición y uso del nitrógeno (estimados a partir de la composición isotópica foliar del nitrógeno: $\delta^{15}\text{N}$). Estos rasgos variaron significativamente entre las especies, y a su vez, estas diferencias cambiaron significativamente a lo largo del tiempo. También se encontraron diferencias significativas entre las especies para otros rasgos foliares, como el área específica foliar (SLA), el contenido relativo en humedad de la hoja (RWC), el contenido en nitrógeno foliar (%N) y la relación entre carbono y nitrógeno de la hoja (C:N), que

también variaron a lo largo de los diferentes momentos de muestreo. La densidad estomática (SD) cambió significativamente entre las diferentes especies, sin embargo, el comportamiento de éstas permaneció constante a lo largo del tiempo. A nivel de las características físicoquímicas del suelo, las especies no mostraron diferencias significativas entre ellas, salvo con la excepción del pH.

Finalmente pudimos confirmar que las especies que coexisten en este frente dunar sujeto de nuestro estudio, difieren ampliamente en varios rasgos funcionales claves para las plantas, relacionados con el uso de recursos esenciales para su desarrollo y supervivencia, como son el agua, el nitrógeno y la radiación, sugiriendo la existencia de una segregación de nichos entre las especies. Los efectos temporales específicos de cada especie en el uso de estos recursos, sugiere una segregación de nichos dependiente del tiempo. A diferencia de lo encontrado en estudios previos en ambientes severos, nuestros resultados muestran un considerable grado de diversidad funcional y complejidad, sugiriendo que el límite de similitud y/o procesos de perturbación pueden ser importantes en la estructuración de las comunidades dunares estudiadas.

En el **capítulo 2**, estudiamos el comportamiento de diferentes especies, que coexisten en un área homogénea y reducida, en cuanto al contenido foliar en agua y pigmentos relacionados con el procesado de la radiación, así como rasgos relacionados con la eficiencia fotoquímica. La teoría ecológica sugiere que en los ambientes severos los principales factores abióticos de estrés pueden actuar a modo de filtros ambientales, reduciendo de ese modo el rango de las posibles estrategias que las plantas pueden adquirir a través de la selección de diferentes rasgos funcionales. Esto conduciría hacia una semejanza funcional de las especies que coexisten en esos ambientes hostiles. Para poder contrastar esa predicción, seleccionamos cinco especies de herbáceas perennes que coexisten en un área del frente dunar de Lariño (Galicia, NO de España). Sobre diferentes individuos de esas especies, se

midieron seis rasgos funcionales de las hojas, claves para el desarrollo de la planta, en tres momentos diferentes a lo largo del año. Esos rasgos funcionales examinados están relacionados concretamente con el estado hídrico de la planta, así como con la capacidad de interceptación y procesado de la radiación de las hojas, que resultan ser dos de los factores de estrés más limitantes de los presentes en los ambientes dunares costeros. Las especies mostraron diferencias significativas para varios índices relacionados con rasgos asociados con la absorción de luz, concretamente con el índice de contenido de clorofila (CHL), el índice de vigor (NDVI), así como la proporción de la radiación absorbida que es procesada por la *vía* fotoquímica (como se expresa mediante el rendimiento cuántico efectivo del fotosistema II (Φ PSII) y mediante el índice de reflectancia fotoquímica (PRI). Para la mayoría de los rasgos funcionales estudiados, el rendimiento relativo entre las especies resultó ser significativamente dependiente del momento en que se realizó la medida. Este estudio encontró una significativa divergencia en los rasgos funcionales de las especies que coexisten en estos ambientes, que no concuerda con los resultados encontrados en otros ambientes de elevada severidad ambiental, donde las especies tienden hacia una convergencia funcional. Nuestros datos aportan soporte experimental a la hipótesis de que no existe una única combinación posible de rasgos funcionales para un ambiente dado, sino que diversos diseños funcionales alternativos, de similar eficacia pueden evolucionar en un mismo ambiente como resultado de la compleja diversidad de interacciones y soluciones de compromiso entre los rasgos. Esto sugiere que los procesos que tienden a producir divergencia, tales como el límite de similitud y los procesos de perturbación, pueden tener un importante papel en la estructuración de la comunidad dunar costera de nuestro estudio. La elevada variabilidad en rasgos funcionales encontrada, sugiere un nivel significativo de diversidad funcional. Además, destaca la gran importancia de la preservación de la composición de especies de los hábitats dunares costeros que se encuentran amenazados, con el objetivo de conservar

procesos funcionales característicos, que pueden ser exclusivos de estos ambientes.

En el **capítulo 3**, estudiamos el comportamiento de dos especies típicas del frente dunar, pero que logran distribuirse más ampliamente tierra adentro, a lo largo de gradientes costa-interior de varios centenares de metros. Se estudió la capacidad de estas especies, en diferentes posiciones a lo largo de los gradientes, para la captación y uso de diferentes recursos, como el agua, el nitrógeno y la radiación, esenciales para el desarrollo y supervivencia de las plantas. Los ecosistemas dunares costeros son ambientes altamente estresantes, sujetos a un alto grado de perturbación, que resulta demasiado hostil para la supervivencia de muchas especies. La dureza de los factores de estrés normalmente disminuye a medida que nos alejamos del frente dunar hacia posiciones más interiores, generando la existencia de gradientes complejos. Este tipo de gradientes proporciona oportunidades únicas para el estudio de respuestas intraespecíficas a variaciones ambientales, y para comprender mejor los efectos de los factores abióticos, que dirigen la evolución de estas comunidades. En el presente estudio, examinamos si las especies que coexisten a lo largo de un gradiente ambiental, supuestamente direccional, perciben los cambios ambientales como no direccionales. También investigamos si la variación de los rasgos funcionales foliares de estas especies están correlacionados con variaciones en los factores ambientales que actúan en este tipo de ambientes, a lo largo de gradientes costa-interior. Finalmente, también analizamos en qué medida difiere el grado de convergencia de los rasgos funcionales de las hojas entre las posiciones de los dos extremos del gradiente. Para poder satisfacer estos objetivos, se recogió información para cuantificar diferentes rasgos funcionales esenciales para la planta, principalmente relacionados con tres de los principales recursos limitantes, como son el agua, el nitrógeno y la radiación. Para ello se emplearon diferentes técnicas, entre la que destacan la discriminación isotópica del carbono y del nitrógeno, la fluorescencia clorofílica y la

reflectancia espectral. También se determinaron varias de las principales características fisicoquímicas del suelo al pie de cada planta, algunas de las cuales pueden actuar como factores de estrés (como el pH o la salinidad) o como importantes recursos limitantes (como el contenido de agua y de nutrientes). Para esto se muestrearon ejemplares de las dos especies seleccionadas (*Eryngium maritimum* y *Pancreatium maritimum*) situadas a diferentes posiciones a lo largo de un gradiente costa-interior de 600 m, en el sistema dunar de Valdoviño (Galicia, NO de España). Los resultados mostraron que las variaciones en la mayoría de las características fisicoquímicas del suelo, a excepción del contenido en nitrógeno, fueron espacialmente dependientes. Las variaciones resultaron ser direccionales únicamente para el contenido hídrico del suelo y la salinidad, cuyos valores decrecieron desde las posiciones más próximas a la costa hacia las más internas. Las especies se diferenciaron a lo largo de las posiciones del gradiente dunar en el contenido de agua del suelo y el pH. Aunque los patrones de variación en los rasgos estructurales fueron espacialmente dependientes, el nivel de dependencia fue diferente para casi todas las posibles combinaciones de especies y rasgos. Las variaciones encontradas para los rasgos funcionales también fueron dependientes de la posición a lo largo del gradiente en que fueron medidas, con excepción del rendimiento cuántico efectivo del fotosistema II (ΦPSII) y la relación isotópica del nitrógeno $^{15}\text{N} / ^{14}\text{N}$. Los patrones de variación fueron direccionales para los índices de reflectancia espectral relacionados con la absorción y el uso de la radiación. Las diferentes especies percibieron las variaciones a lo largo de los gradientes, y respondieron a éstos, de diferente manera. Esto nos indica una excesiva simplificación en los estudios previos que han pretendido determinar los principales factores ambientales que puedan explicar la zonación de las especies a lo largo de los gradientes dunares.

En el **capítulo 4**, estudiamos tanto la variabilidad interespecífica como espacial en las respuestas de especies de ecosistemas dunares costeros a la

radiación. La radiación tiene un efecto determinante en el desarrollo de la estructura y el funcionamiento de los ecosistemas dunares costeros, que se caracterizan por poder alcanzar elevados niveles de radiación, bien sea por incidencia directa como por reflexión. El alto grado de variación mostrado por este recurso demanda un elevado nivel de plasticidad en el sistema fotosintético de las plantas. Esta plasticidad fue estudiada en condiciones de campo, mediante la aplicación de diversas técnicas como el análisis de curvas de respuesta a la radiación, curvas de recuperación en oscuridad, así como el estudio de otros índices como el rendimiento cuántico máximo y efectivo del fotosistema II (F_v / F_m y Φ_{PSII} respectivamente), o como la disipación fotoquímica y no fotoquímica (q_P y q_N respectivamente) de la radiación, todos ellos basados en parámetros de la fluorescencia clorofílica. Este estudio se centra, por un lado, en cómo especies que coexisten en el frente dunar, con contrastadas morfologías foliares y formas de crecimiento, difieren en sus eficiencias fotoquímicas en respuesta a cambios rápidos en las condiciones de iluminación. Por otro lado, también estudiamos cómo las variaciones en la intensidad de los factores de estrés, que actúan a lo largo de un gradiente ambiental costa-interior, afectan a las respuestas de *Pancreatum maritimum* a la radiación. También examinamos la variabilidad temporal de las respuestas de las plantas a la radiación, comparando su comportamiento entre dos estaciones contrastadas (verano e invierno). Las plantas del frente dunar mostraron diferencias en sus estrategias del uso de la radiación, pero sin embargo mostraron patrones de recuperación en oscuridad muy similares. Las diferencias entre las especies, para las reacciones fotoquímicas del fotosistema II, cambiaron estacionalmente. *Matthiola sinuata* puede ser descrita como una especie especialista de invierno, *Eryngium maritimum* como una especie especialista de verano, mientras que *Euphorbia paralias* y *Pancreatum maritimum* son consideradas especies de carácter más generalista, debido a que mantienen unas eficiencias similares a lo largo de ambas estaciones. Diferencias estacionales en su rendimiento relativo pueden estar permitiendo

la coexistencia de las especies. Algunos de los rasgos medidos del comportamiento fotoquímico de *P. maritimum* cambiaron significativamente a lo largo del gradiente dunar. Contrariamente a lo esperado, otros rasgos no variaron de la forma esperada, o directamente no cambiaron significativamente.

Los cambios en la eficiencia del fotosistema II de *P. maritimum* no fueron consistentes con los supuestos cambios direccionales en la severidad de los factores de estrés. El conjunto de factores ambientales que influyen en el rendimiento de las plantas, variable espacial y temporalmente, originó patrones de variación impredecibles a lo largo del gradiente, en rasgos relacionados con los procesos fotoquímicos del fotosistema II. Los patrones de recuperación en condiciones de oscuridad fueron muy similares, con independencia de las condiciones experimentales (especies y posiciones en los gradientes). Esta circunstancia sugiere que puede haber presiones evolutivas que estén actuando para mantener unos mecanismos de recuperación muy similares entre las plantas.

En el **capítulo 5**, comparamos la variabilidad de los sexos de *Honckenya peploides* en relación a la disponibilidad hídrica y a la distribución y acumulación de biomasa en las diferentes estructuras. Las plantas dioicas a menudo muestran diferencias dependientes del sexo en el crecimiento y en la distribución de biomasa a las distintas estructuras de la planta. Estas diferencias se explican como consecuencia de las diferentes funciones reproductivas desarrolladas por los distintos sexos. Pruebas experimentales apoyan consistentemente que las hembras llevan a cabo una mayor inversión en estructuras reproductivas. Diferencias entre los sexos en la distribución de los recursos puede determinar el rendimiento de cada uno de los sexos en distintos hábitats y por tanto podría explicar la segregación espacial descrita en muchas especies dimórficas. En nuestro caso, investigamos el dimorfismo en patrones estacionales de distribución de biomasa en la herbácea perenne subdioica *Honckenya peploides*, una especie que crece en la duna embrionaria

(las formaciones más incipientes de los ambientes dunares) y que muestra segregación espacial de los sexos en la localidad de estudio seleccionada. Además, también estudiamos el contenido hídrico de suelos de lugares donde crecen los machos y de lugares donde crecen las hembras, en diferentes momentos a lo largo de la estación de crecimiento. En este trabajo comparamos los patrones estacionales de disponibilidad de agua en el suelo y distribución de la biomasa entre las diferentes estructuras, a lo largo de dos años consecutivos. Este proceso se realizó en machos y en hembras de *H. peploides*, mediante muestreo de suelo y de muestras de planta en poblaciones naturales de esta especie. Se estudiaron perfiles verticales de biomasa y contenido hídrico del suelo mediante la extracción de muestras de suelo, en hábitats de machos y de hembras, a diferentes profundidades.

Las plantas de los distintos sexos de *H. peploides* se diferenciaron en sus patrones estacionales de distribución de la biomasa hacia estructuras reproductivas. Los machos invirtieron hasta dos veces más que las hembras, en reproducción en momentos más tempranos, a lo largo de la estación de crecimiento pero la situación se invirtió al ir progresando la estación. No se encontraron diferencias entre los sexos en cuanto a inversión en biomasa aérea, pero la distribución de biomasa hacia estructuras subterráneas mostró diferencias en profundidad entre plantas macho y hembras, siendo las hembras quienes normalmente mostraron una mayor acumulación de biomasa en estructuras subterráneas. Además las plantas macho y hembras de esta especie mostraron diferentes patrones en el contenido de agua en el suelo en el que crecieron. Cuando se encontraron diferencias significativas (normalmente a nivel de las capas superiores del suelo), fueron las hembras quienes mostraron los mayores contenidos de agua. Los distintos momentos de inversión en reproducción que muestran los sexos, y la diferente disponibilidad y uso de recursos del suelo (principalmente agua), son factores que probablemente compensen los costes de la inversión en reproducción en el crecimiento aéreo en machos y hembras de *H. peploides*. Nuestros resultados sugieren que los

patrones de segregación espacial de los sexos, observados en *H. peploides*, puede contribuir a maximizar el crecimiento y reproducción de cada uno de los sexos.

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