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Balanço de carbono em ecossistemas mediterrânicos

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Tese apresentada para obtenção do grau de doutor em
Engenharia Florestal e dos Recursos Naturais

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LISBOA
2013

Resumo

As florestas desempenham um papel importante na mitigação das alterações climáticas porque sequestram e armazenam o dióxido de carbono (CO_2), um dos principais gases com efeito de estufa. O objectivo desta dissertação foi investigar o balanço de carbono nas florestas nas suas principais componentes: árvores, arbustos e solo.

Apresentam-se métodos para estimar o reservatório de carbono nas árvores em povoamentos de pinheiro manso no sul de Portugal. Os modelos alométricos, factores de conversão e de expansão de biomassa apresentados permitem obter estimativas de carbono independentemente dos dados de inventário existentes sobre os povoamentos.

O subcoberto arbustivo de um montado de sobro contribuiu 20% para a assimilação total de carbono do ecossistema durante o Verão/Outono 2011. Provou-se que as espécies arbustivas que colonizaram naturalmente o subcoberto utilizam estratégias diferentes para aproveitar os recursos ambientais limitantes como a água e a luz.

O efluxo de CO_2 dos solos, resultado da respiração dos microorganismos e das raízes, é semelhante entre florestas, matagais e pastagens. A humidade do solo, mais do que a temperatura, é determinante neste processo. São apresentados modelos empíricos, calibrados e validados recorrendo à estatística Bayesiana, que permitem estimar mensalmente a respiração do solo em pastagens da região mediterrânica.

Palavras chave: balanço de carbono, mediterrânico, *Pinus pinea*, modelos alométricos, respiração do solo, estatística Bayesiana, Cistus, Ulex, *eddy*-covariância, *upscaling*

Title: Carbon balance in Mediterranean ecosystems

Abstract

Forests play an important role in climate change mitigation as they sequester and store carbon dioxide (CO_2) from the atmosphere. The aim of this thesis was to investigate forest carbon balance in its main compartments: tress, understory and soils.

We present methods to estimate carbon stock in biomass of stone pine stands in south Portugal. Allometric models, as well as conversion and expansion factors were presented allowing the quantification of stand carbon stocks irrespective of the inventory base information available for the site.

The undercanopy vegetation of a cork oak *montado* represented 20% of the total carbon assimilated by the ecosystem during summer/autumn 2011. It was proven that the vegetation mosaic that naturally colonized the understory have contrasting strategies to efficiently use the limited environmental resources available like water and light.

Soil CO_2 efflux, resulted from plants and microorganisms respiration, is similar between forests, shrublands and grasslands. Soil moisture, more than temperature, is determinant in this process. We present several empirical models, validated and calibrated using the Bayesian statistics, which allows estimating monthly soil respiration in grasslands for the Mediterranean region.

Keywords: carbon balance, mediterranean, *Pinus pinea*, allometric models, soil respiration, Bayesian statistic, Cistus, Ulex, eddy-covariance, upscaling

Agradecimentos

A realização deste trabalho foi possível com o apoio e contributo de pessoas e entidades, a quem desejo manifestar o meu agradecimento.

Ao professor João Santos Pereira quero agradecer os valiosos conhecimentos que me transmititu ao longo de 12 anos, pelo despertar da curiosidade pelo tema do carbono e das florestas, e pela liberdade com que me deixou decidir o meu percurso científico.

Quero manifestar o meu especial agradecimento às seguintes pessoas que, não constando na co-autoria, contribuiram para que o artigo: *Biomass allometry and carbon factors for a Mediterranean pine (Pinus pinea L.) in Portugal* se concretizasse. Agradeço à Engª Mafalda Evangelista, à Engª Marta Bastos e ao Engº Manuel Tuna da Herdade de Monte Novo de Palma pelo apoio no trabalho de campo assim como às equipas de campo da AFLOPS pelo trabalho profissional prestado durante o abate das árvores. Quero agradecer ao Engº Luís Unas pelo apoio na elaboração da candidatura do projecto AGRO – acção 8.1 com o título *O sequestro de carbono e a gestão florestal sustentável no sul de Portugal*, sem o qual este trabalho não teria sido possível.

Quero agradecer aos meus colegas do departamento por quem tenho a maior admiração. Ao Filipe Costa e Silva, à Helena Santos, à Ana Rodrigues, ao António Correia, ao Alexandre Correia, à Marta Carneiro, à Carla Faria, à Sofia Cerasoli, à Teresa Sampaio, à Márcia Santos, à Raquel Lobo do Vale, à Marjan Jongen, ao Stephan Unger e ao Francesco Minnuno pelo óptimo ambiente de trabalho, pela troca de ideias, pelo apoio nas fases mais complicadas e por me acompanharem nesta árdua tarefa de concluir um doutoramento. Um agradecimento especial à professora Helena Almeida, à dona Lurdes e à dona Madalena por todo o apoio e amizade.

Agradeço à minha família, em especial ao meu marido Jorge, aos meus pais Armindo e Luisa e à minha irmã Rita por toda a ajuda. Dedico este trabalho aos meus filhos Natália e Artur e à minha avó Nela.

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CAPÍTULO IV

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1. Ciclo global de carbono e importância dos ecossistemas terrestres

O objectivo desta dissertação foi estudar o balanço de carbono em ecossistemas florestais através de três estudos independentes, centrados nos principais reservatórios de carbono das florestas: árvores, arbustos e solo. O estudo engloba metodologias de avaliação específicas para cada reservatório mas com um fio condutor comum que é produzir informação que permite uma avaliação mais rigorosa do balanço de carbono nas florestas mediterrânicas nestes três reservatórios.

O ciclo de carbono consiste na transferência do dióxido de carbono (CO_2), via combustão, respiração ou reacções químicas para a atmosfera ou para o mar e a sua reintegração na matéria orgânica, via assimilação fotossintética. O ciclo global do carbono tem sido alterado pelas emissões antropogénicas de CO_2 resultantes de alterações de uso do solo, nomeadamente desflorestação, e da queima de combustíveis fósseis, que só são parcialmente compensadas pela absorção pelos oceanos e pela vegetação terrestre, levando à acumulação de CO_2 na atmosfera.

Esta acumulação de gases com efeito de estufa (GEE) na atmosfera está na origem de uma alteração do balanço radiativo terrestre. A consequência é um aumento das temperaturas médias à superfície. Note-se que sem a protecção oferecida pelos GEE, a temperatura média da atmosfera seria próxima dos -18ºC em lugar dos actuais 15ºC. O que há de verdadeiramente inédito na situação presente é a elevada taxa de variação da concentração de CO_2 na atmosfera. Nas últimas décadas, a taxa média anual de aumento foi cem vezes superior aquela que se registou nas transições recentes dos períodos glaciares para os períodos interglaciares (IPCC, 2001). Tudo indica que a concentração deste gás deverá continuar a aumentar durante as próximas décadas devido à forte dependência da economia mundial dos combustíveis fósseis. Por outro lado, mesmo que ocorresse agora uma redução drástica das emissões, por exemplo através da revisão das políticas energéticas, os efeitos continuariam a fazer-se sentir por muitas décadas devido ao elevado tempo de residência do CO_2 na atmosfera (cerca de 100 anos). Esta interferência no clima do planeta fragiliza o equilíbrio natural dos ecossistemas. É fundamentalmente a rapidez com que este fenómeno tem vindo a ocorrer, que não é compatível com a velocidade de ajustamento natural dos ecossistemas.

O oceano constitui não só o maior reservatório mas também um dos principais sumidouros de CO_2 da atmosfera absorvendo cerca de 30% do carbono de origem antropogénica emitido anualmente para a atmosfera (Sabine *et al.*, 2004). Este sequestro resulta não só da actividade de sequestro biológico pelas plantas aquáticas, mas também da solubilização do CO_2 na água que depende da concentração de CO_2 na atmosfera e da temperatura da água. Um dos *feedbacks* negativos do aumento das temperaturas é

precisamente uma diminuição da solubilização deste gás nos oceanos. Interferências no sistema de circulação da atmosfera afectam também as correntes marítimas, deslocando camadas profundas de água carregadas de carbono fóssil para a superfície (Schuster e Watson, 2007). Os oceanos, outrora assumidamente sumidouros de carbono, nem sempre funcionam como tal. Um estudo desenvolvido por Le Quere *et al.* (2007) refere por exemplo, que alterações observadas da circulação dos ventos no hemisfério sul ocasionaram uma redução significativa da capacidade de sumidouro dos oceanos entre 1981 e 2004.

A dinâmica do ciclo de carbono é muito variável, quer no espaço quer no tempo, sendo controlada fundamentalmente pelos sistemas que têm capacidade de o trocar activamente com a atmosfera, como é o caso da vegetação e do solo. Estima-se que cerca de 2100 Gt de carbono se encontre armazenado nos ecossistemas terrestres, valor quase três vezes superior ao que existe actualmente na atmosfera (Trumper *et al.*, 2009). Estes ecossistemas, dos quais se destacam as florestas, assumem assim um papel de relevo na mitigação dos impactos das alterações climáticas, não só por trocarem activamente carbono com a atmosfera, mas também pela capacidade de reterem carbono na biomassa perene e no solo tal como veremos a seguir.

A fotossíntese que ocorre nas plantas é responsável pela retenção de carbono atmosférico no material vegetal e na matéria orgânica no solo. Por esse motivo, ecossistemas com grande biomassa e com o solo pouco perturbado, como as florestas, retêm o carbono numa escala temporal muito maior, na ordem de décadas e séculos. O balanço de carbono nas florestas corresponde à diferença entre os ganhos pela fotossíntese e as perdas pela actividade respiratória de toda a cadeia trófica do ecossistema. A assimilação fotossintética do carbono corresponde à quantidade de CO_2 que é fixado pela vegetação (produtividade primária bruta, PPB) e que pode ser acumulado a longo prazo no ecossistema (biomassa perene e matéria orgânica do solo). Durante este processo ocorrem perdas de CO_2 resultantes da respiração das plantas (Respiração autotrófica, Ra) e dos animais e microorganismos do solo (Respiração heterotrófica, Rh) (Figura 1). O balanço anual de carbono de um ecossistema florestal é, quase sempre, positivo porque descontando as perdas resultantes da respiração e mortalidade natural, o carbono é acumulado nos tecidos vegetais de longa duração e no solo (Produtividade líquida do ecossistema, PLE). Importa ainda referir que, numa avaliação do balanço de carbono à escola regional, há que considerar as perdas devido aos cortes e exploração de produtos florestais e ainda e as emissões decorrentes dos incêndios florestais. Obtém-se assim a produtividade líquida do bioma, PLB.

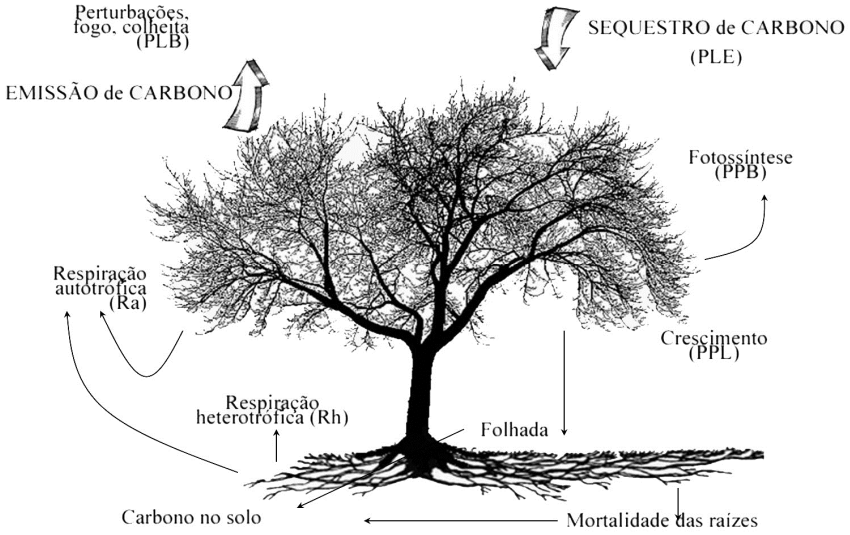

Figura 1 – Principais fluxos de carbono na floresta, entre a biomassa e o solo.

As folhas, raízes finas, frutos e outros órgãos sésseis, contribuem para incrementar o carbono nos solos. Porém, parte deste carbono é perdido por respiração devido à actividade microbiana. É fundamentalmente no horizonte orgânico que ocorrem os principais efluxos de CO_2 anuais para a atmosfera (Ver Capítulo IV), enquanto nas camadas mais profundas do horizonte mineral, ocorre a acumulação de formas químicas de carbono recalcitrante. Esta libertação de carbono depende não só de variáveis climáticas, que determinam a actividade dos microorganismos e as taxas de produção e decomposição de matéria orgânica, mas também das propriedades dos solos, que afectam a mineralização das componentes orgânicas pelas interacções físicas e químicas com os minerais do solo. Janssens *et al*. (2001) estimou, através de um estudo comparativo em 18 ecossistemas europeus, que aproximadamente 80% do carbono assimilado pela vegetação viva é perdido através de respiração. Desta fracção, cerca de 70% corresponde a respiração do solo. Em resumo, para uma melhor compreensão dos processos associados à dinâmica do balanço de carbono nas florestas é fundamental que esta componente da respiração seja levada em consideração.

Há que distinguir o conceito de reservatório do de sequestro de carbono. O reservatório de carbono corresponde à quantidade de biomassa (em carbono) que uma floresta armazena, quer nas suas componentes perenes (tronco, raízes, ramos), quer no solo e traduz um valor por unidade de área. O sequestro de carbono é uma variável dinâmica e corresponde a um armazenamento líquido por unidade de tempo. Com efeito, uma floresta pode ser um grande reservatório de carbono mas contribuir pouco enquanto sequestradora de carbono.

A mais recente estimativa do reservatório de carbono nas florestas é de 861 ± 66 Pg de carbono sendo que aproximadamente 42% (363 ± 28 Pg C) se encontra na biomassa viva (Pan *et al.*, 2011). É nos solos das

florestas boreais que reside o maior reservatório de carbono (aproximadamente 23% do total dos solos) da biosfera terrestre, aprisionado nos horizontes orgânicos de solos congelados (*permafrost*) (Figura 2). Aqui, as baixas temperaturas são o factor limitante à actividade decompositora dos microorganismos do solo, dando origem a uma taxa de deposição de material orgânico superior à taxa de decomposição. Este carbono é com efeito, bastante sensível às temperaturas e o aquecimento global tem conduzido a um aumentado da libertação de CO_2 e outros GEE para a atmosfera (Goulden *et al.*, 1998; Karhu *et al.*, 2010; Deluca e Boisvenue, 2012). Se por um lado esta aceleração do ciclo de nutrientes tem beneficiado a produtividade vegetal nestas regiões (Welp *et al.*, 2007; Trucco *et al.*, 2012), de acordo com alguns autores, seriam necessários aumentos de produtividade da vegetação de cerca de 100-120% para compensar esta libertação de carbono devido à decomposição (Karhu *et al.*, 2010).

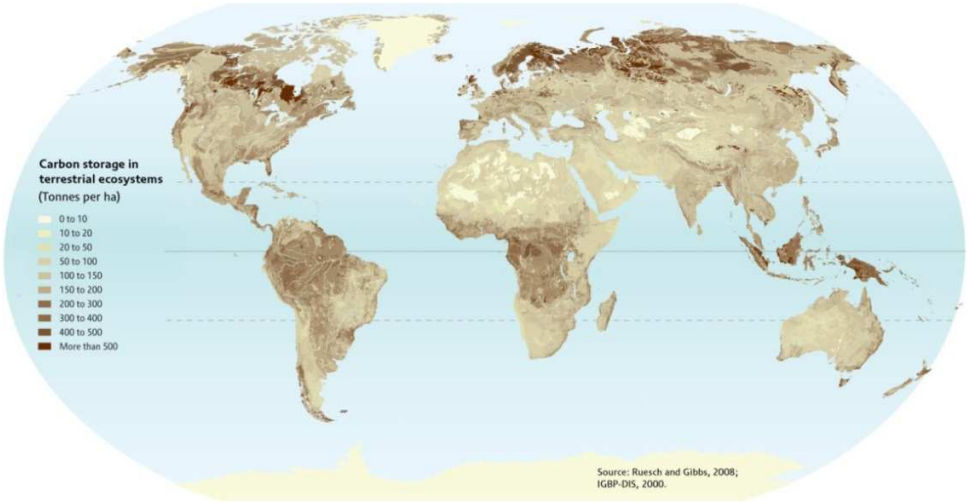

Figura 2 – Reservatório global de carbono na biomassa e no solo até 1 m de profundidade (Riccardo Pravettoni, UNEP/GRID - Arendal).

No que se refere ao sequestro de carbono, as florestas contribuem com uma remoção de CO_2 da atmosfera na ordem das 1.8 Pg C por ano correspondendo a 40% das emissões anuais antropogénicas de GEE (Stephens *et al.*, 2007). As florestas tropicais e subtropicais são as que mais contribuem para este valor. Beer *et al.* (2010) estimou, através do método do fluxo turbulento, uma contribuição destes ecossistemas na ordem dos 60% em relação ao total do carbono assimilado globalmente pelos florestas. A conservação destes ecossistemas é, só pelo objectivo de regular o clima do planeta, fundamental.

As metodologias de avaliação do sequestro de carbono variam consoante o tipo de medições e escalas espaciais que se pretendem medir. Podem basear-se em inventários florestais, em modelos de crescimento ou em medição dos fluxos de carbono. Nos inventários florestais, o sequestro é calculado com base em estimativas da variação do reservatório de carbono entre dois períodos temporais ou então com base em estimativas do reservatório e do crescimento da floresta. Nestes inventários recorre-se à medição das dimensões da árvore em parcelas representativas de uma área ou região, aplicando-se subsequentemente

modelos alométricos para estimar a biomassa (em matéria seca ou carbono) na parte aérea e/ou radicular nas árvores dessas parcelas (ver Capítulo III). Este método não se restringe à medição das árvores, podendo ser utilizado também para quantificar a biomassa no subcoberto e dos solos. Os modelos para quantificar o sequestro de carbono podem assumir várias estruturas dependendo dos dados a partir dos quais eles são desenvolvidos. Permitem simular a evolução da floresta ao longo do tempo, podendo acomodar o efeito do clima sobre o crescimento da vegetação assim como intervenções culturais, impactes de pragas e doenças ou do fogo. Podem ser do tipo estatístico, isto é, baseados em métodos estatísticos (regressões) que recorrem a um grande número de medições das árvores ao longo do tempo, ou de base processual, baseando-se em processos fisiológicos de resposta das plantas a variações dos factores ambientais. Finalmente, uma medição relativamente directa do sequestro de carbono do ecossistema, recorre ao método da covariância de fluxos turbulento (*Eddy-covariance*) que permite monitorizar variáveis meteorológicas assim como os fluxos de carbono e água entre o ecossistema e a atmosfera. Este é um método que reúne actualmente grande consenso científico e tem sido aplicado em larga escala em todo o globo para rastrear o balanço de água e carbono em inúmeros ecossistemas terrestres.

Existe uma interacção entre o ciclo de carbono e outros ciclos biogeoquímicos, como o de nutrientes e o hidrológico, que estão em aparente equilíbrio. Porém, a ciência das alterações climáticas conclui que, acima de tudo, estas interacções são complexas e intrincadas. Particularmente sensíveis são os ecossistemas mediterrânicos e daí o enorme interesse no seu estudo.

1.1. Ecossistemas florestais da região mediterrânica

Os ecossistemas mediterrânicos cobrem pouco mais de 2% da superfície terrestre. Pela sua pouca expressão a nível global, os inventários de reservatórios de carbono ou produtividade primária são, na generalidade dos casos, agrupados em outras classificações biogeográficas mais abrangentes como o bioma da região temperada ou savanas semi-tropicais. De facto, e de acordo com a pesquisa bibliográfica realizada, não foram encontradas estimativas de balanço de carbono gerais para a região mediterrânica. Sendo assim, comparações entre biomas tornam-se mais complicadas.

Na Figura 2 observa-se que o reservatório de carbono no mediterrânico varia entre 50 e 400 toneladas por hectare. Produtividades líquidas tão discrepantes parecem ser explicadas, em parte, pela multiplicidade de usos do solo, por vezes coexistindo no mesmo local, e sobretudo pela disponibilidade de água. No mediterrânio, a produtividade vegetal é uma variável muito bem correlacionada com a disponibilidade de água no solo, mais do que com a temperatura como acontece com a maioria dos ecossistemas da região

temperada e boreal. A variação espacial dos reservatórios de carbono depende da precipitação total anual, que condiciona o tipo de ocupação vegetal e a quantidade máxima de "área fotossintética" que um solo pode sustentar (Figura 2). Já a variabilidade intra-anual da produtividade primária está relacionada com a sazonalidade da precipitação e uma distribuição homogénea das chuvas, sobretudo na Primavera (Figura 3). A duração da estação de crescimento das plantas é, com efeito, o factor que determina, em última instância, o balanço de carbono anual de um ecossistema (Baldocchi, 2008).

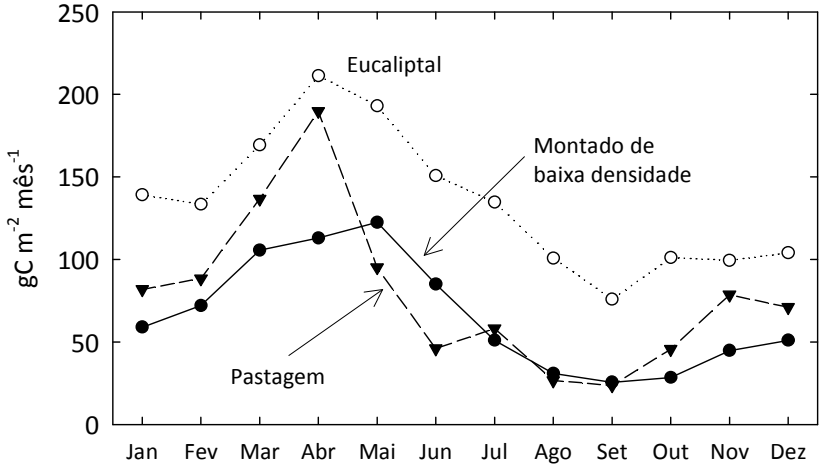

Figura 3 – Produtividade primária bruta (média mensal para um ano com precipitação normal) em 3 ecossistemas contrastantes da região mediterrânica estimada pelo método do fluxo turbulento num eucaliptal, montado de baixa densidade e numa pastagem mediterrânica (adaptado de Pereira *et al*, 2007.

Em contrapartida, a precipitação concentrada no tempo não é geralmente bem aproveitada pelas plantas devido à fraca capacidade de retenção dos solos para a água, inviabilizando um eficiente armazenamento desta em profundidade. Chuvas esporádicas no meio da estação seca não apresentam também qualquer vantagem para as plantas porque, normalmente humedecem apenas as camadas superficiais do solo e têm pouco efeito sobre a fotossíntese. Na realidade, estas chuvas nos Verão estimulam antes a actividade microbiana do solo causando uma emissão significativa de carbono para a atmosfera – *Birch effect* (Jarvis *et al.*, 2007; Unger *et al.*, 2012, ver também Capítulo IV). Em ecossistemas de baixa produtividade, como é o caso dos montados da região interior do país, estes fenómenos podem converter só por si, o ecossistema numa fonte anual de carbono para a atmosfera (Pereira *et al.*, 2007).

As espécies lenhosas mais bem sucedidas na região mediterrânica estão extraordinariamente bem adaptadas à sobrevivência durante o Verão. Nesta altura do ano, a evapotranspiração é com frequência superior à precipitação constituindo um factor adicional limitante à produtividade. Após o pico de produtividade na Primavera, a fotossíntese é reduzida para valores mínimos de modo a reduzir a transpiração (Figura 3) compensando ao mesmo tempo os custos em carbono associados à manutenção e crescimento dos tecidos vivos (ver Capítulo III). As plantas recuperam somente após as chuvas consecutivas

de Outono (Figura 3). Esta regulação eficiente da fotossíntese e das perdas de água resultam do elevado controlo estomático explicado, em parte, pelo acesso das raízes à água das camadas mais profundas e húmidas do solo (David *et al.*, 2007). Os arbustos evoluíram igualmente com grande sucesso, apresentando adaptações quer à seca (ver Capítulo III), quer ao fogo (Scarascia-Mugnozza *et al.*, 2000). As folhas pequenas e esclerificadas reduzem as perdas de água no Verão. Muitas apresentam adaptações morfológicas que minimizam o colapso do aparelho fotossintético provocado pela combinação de vários *stresses* no Verão, como é o excesso de radiação e o deficit hídrico (Werner *et al.*, 2001). A capacidade de germinação de sementes estimulada pelas temperaturas elevadas, a regeneração por toiça, rebentos basais ou rizomas após o fogo, são atributos que sugerem uma evolução especializada e eficiente a um clima muito próximo do deserto.

Já as comunidades de plantas herbáceas, por outro lado, tendem a evitar a secura estival. São típicas de climas temperados, com ciclos de vida anuais. Nesta, a produtividade acompanha a precipitação sazonal com um pico na primavera, senescendo antes do estabelecimento da secura estival. Ainda assim parecem bem adaptadas a uma certa irregularidade na precipitação característica do clima mediterrânico, suportando períodos relativamente longos de deficiência hídrica mesmo durante a estação de crescimento (Jongen *et al.*, 2013).

Os solos da região mediterrânica são naturalmente pobres. Uma vez que a produtividade é baixa a incorporação de material orgânico é pouca. É frequente a ausência de horizontes orgânicos, que estão normalmente circunscritos às regiões de maior pluviosidade, tal como se pode observar na Figura 4 para o caso português.

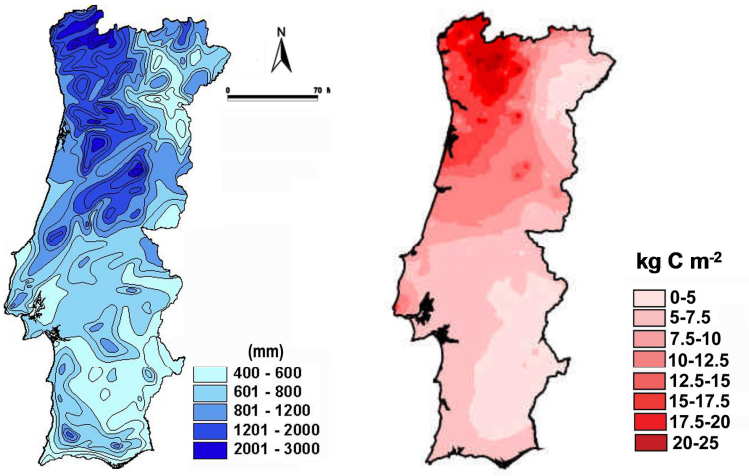

Figura 4 – Distribuição espacial da precipitação média anual (esquerda) e da quantidade de carbono no solo até à profundidade de 30 cm (adaptado de Madeira *et al.*, 2004).

Para compensar esta carência de nutrientes dos solos florestais, que é comum na generalidade dos solos florestais, as árvores evoluíram no sentido de limitar esta perda reciclando os nutrientes internamente. Assim, antes da queda das folhas, ocorre uma translocação de nutrientes minerais absorvidos durante o período de crescimento para as partes perenes da planta. Estes são depois remobilizados para os tecidos em crescimento aquando do início da estação de crescimento (Cerasoli *et al.*, 2004). Uma particularidade dos solos de climas semi-áridos é com efeito, a sua propensão à degradação e desertificação. A fraca cobertura vegetal torna-os mais expostos à erosão e o fogo fomenta ainda mais os estágios avançados de degradação. O reservatório de carbono nos solos é portanto baixo quando comparado com outros ecossistemas florestais do planeta.

A produtividade potencial das zonas mediterrânicas é porém enorme, sobretudo nas regiões onde o *deficit* hídrico não é muito acentuado. Isto deve-se fundamentalmente ao elevado número de horas e dias de luz por ano associado a temperaturas amenas. Os valores de produtividade líquida do ecossistema medidos pelo método do fluxo turbulento num eucaliptal de produção da região de Pegões com 1110 árvores/ha (Pita *et al.*, 2011, Figura 3) são um bom exemplo deste elevado potencial. Neste estudo, que monitorizou o ecossistema durante 8 anos, a produtividade média anual obtida (-623 $gCm^{-2}ano^{-1}$)[(1)], aproximou-se dos valores máximos medidos numa rede de 124 ecossistemas do globo (Baldocchi, 2008). Um estudo mais recente utilizando a mesma técnica mas que monitorizou um montado de sobreiro certificado para produção de cortiça na região de Coruche com 170 árvores/ha, indica que o ecossistema foi igualmente um sumidouro de carbono relevante e sobretudo resiliente a flutuações interanuais da precipitação com uma média de -300 $gCm^{-2}ano^{-1}$ (n=3). A produtividade decresce significativamente em regiões com moderado/acentuada deficiência hídrica. Na região de Évora monitorizou-se um montado de azinheira de baixa produtividade (50 árvores/ha) e observou-se que o ecossistema oscilou entre uma fonte e um sumidouro anual de carbono para a atmosfera consoante se verificaram anos menos ou mais pluviosos, respectivamente.

As espécies lenhosas do clima mediterrânico são, do ponto de vista de alteração climática, particularmente interessantes não só para a ciência mas para a silvicultura do futuro (Capítulo II). Como vegetam em zonas geográficas limitantes em água e nutrientes, poderão ser particularmente interessantes em futuras arborizações de regiões onde se prevê um clima mais quente e seco. Apesar da notável adaptação e resiliência das plantas deste clima à variabilidade intra e interanual das condições meteorológicas, aos solos hostis e à secular presença humana, que respostas esperar num cenário de alteração de clima?

[1] valores negativos representam sequestro de carbono

1.2. O clima e impactes na fisiologia das plantas da região mediterrânica

Primeiro importa perceber quais os efeitos de um aumento da concentração do CO_2 na fisiologia das plantas. Um aumento deste gás na atmosfera poderá proporcionar ganhos em produtividade no curto prazo, mas o mesmo poderá não acontecer no longo termo. Sendo o CO_2 o substrato para a fotossíntese, o aumento da sua concentração na atmosfera pode ter um efeito positivo no crescimento das plantas. Quando uma folha é exposta a uma atmosfera de CO_2 elevado, as taxas fotossintéticas aumentam porque a actividade e eficiência da enzima rubisco[2] é estimulada pela presença de maiores concentrações de substrato (Farquhar *et al.*, 2001). Esta enzima cataliza duas reacções: a de carboxilação, com fixação de carbono, e de oxigenação, com libertação de CO_2 (fotorespiração). Estas reacções competem entre si. Ao aumentar a pressão parcial de CO_2 nos espaços intercelulares das folhas, a reacção de carboxilação é favorecida, ou seja mais carbono é fixado e, portanto, maior é a produtividade. Embora uma atmosfera rica em CO_2 tenha um efeito estimulante no crescimento (Idso e Kimball, 1993; Duff *et al.*, 1994; Palanisamy, 1999; Roden *et al.*, 1999) este efeito é apenas de curta duração. Isto acontece porque as plantas que vivem em ambientes ricos em CO_2 tendem a ajustar as taxas de fotossíntese para níveis mais baixos do que seria de esperar pela resposta de curto prazo. Este processo denominado *down-regulation* (atenuação regulada da fotossíntese) foi observado em florestas da Europa (Roden e Ball, 1996a; Saxe *et al.*, 1998; Medlyn *et al.*, 1999) e está relacionado com uma regulação bioquímica do aparelho fotossintético devido à presença de concentrações elevadas de CO_2. Uma revisão dos resultados da investigação feita em povoamentos adultos de várias espécies arbóreas em meios enriquecidos em CO_2 durante períodos superiores a quatro anos, não mostrou alterações significativas de produtividade, em cotejo com as plantas numa atmosfera com CO_2 actual, principalmente quando outros factores eram limitantes (Asshoff *et al.*, 2006). A conclusão semelhante chegaram (Maroco *et al.*, 2002) ao estudarem a resposta de sobreiros mantidos numa atmosfera enriquecida em CO_2 por quatro anos. Só com abundante adubação azotada houve resposta ao enriquecimento do ar em CO_2.

Que respostas esperar num clima mais quente e seco? Na presença de temperaturas elevadas a afinidade da rubisco para o O_2 decresce mas não tão rapidamente como para o CO_2, resultando numa diminuição da taxa de fotossíntese (isto é, fotossíntese – fotorrespiração) devido ao aumento relativo da reacção de oxigenação. As taxas de respiração também aumentam com a temperatura devido à sensibilidade das enzimas, renovação de proteínas e necessidade energética das reacções de respiração. Este é um dos

[2] A rubisco, abreviatura de ribulose-1,5-bifosfato-carboxilase/oxigenase, é a enzima responsável pela fixação do CO_2 atmosférico, catalizando o primeiro passo do Ciclo de Calvin. É também a proteína mais abundante nos tecidos vegetais verdes, e por conseguinte, de todo o planeta.

principais factores responsáveis pela redução da produtividade num cenário de aquecimento, aumentando a perda de carbono durante o Verão. Em contrapartida, o aquecimento no Outono e Inverno promoverá uma maior produtividade devido ao prolongamento do período "verde", isto é, do tempo entre o abrolhamento na Primavera ou paragem do crescimento no Verão.

A água é um factor determinante no sucesso do estabelecimento de novas plantas e, a par com a nutrição mineral, influencia a performance desta ao longo da sua vida. Os efeitos da limitação de água na produtividade é já conhecido mas serão idênticas as respostas se as plantas crescerem com CO_2 elevado? Estudos em espécies lenhosas em que se controlaram os factores água e CO_2 por períodos superiores a uma estação de crescimento, sugerem que as plantas que crescem em ambientes ricos em CO_2 utilizaram água de forma bastante mais eficiente quando este factor é limitante em comparação com as plantas que crescem a CO_2 ambiente (Conroy *et al.*, 1990; Chaves e Pereira, 1992; Liang *et al.*, 1995; Roden e Ball, 1996b; Medlyn *et al.*, 2001; Centritto *et al.*, 2002; Johnson *et al.*, 2002; Kupper *et al.*, 2006). Parece portanto possível que nas regiões com deficit hídrico moderado no futuro, o aumento da concentração de CO_2 na atmosfera possa compensar parcialmente os efeitos negativos da falta de água, mesmo que ocorra *down-regulation* da fotossíntese.

Do ponto de vista ecológico, um aumento das temperaturas, para além das perdas de produtividade primária, tenderá a aumentar substancialmente o risco meteorológico de incêndio que é actualmente um dos maiores obstáculos à gestão sustentável das florestas mediterrânicas. O prolongamento da estiagem e o aumento da frequência de ondas de calor no Verão poderão exacerbar o risco de incêndios florestais. Esta colheita precoce do material lenhoso reduz substancialmente o potencial de sequestro de carbono no território nacional, não só pela perda de área de floresta, mas também pela emissão de GEE durante a combustão. Em 2003, ano em que arderam cerca de 420 mil hectares de área arborizada em Portugal com libertação de 7415 Gg CO_2 eq. para a atmosfera, as emissões de GEE totais em Portugal aumentaram 10% (Portuguese Environmental Agency, 2012).

O conhecimento destes processos é necessário quando integrado numa abordagem de modelação. Embora não seja possível descrever completamente os impactes do clima na complexa e intrincada teia de processos que ocorrem na natureza, os modelos continuam a ser ferramentas essenciais no processo de tomada de decisões. Apesar da esmagadora maioria dos processos de resposta das plantas a alterações ambientais estarem razoavelmente bem descritos para a região mediterrânica, a modelação do balanço de carbono e da água ao nível do ecossistema apresenta sucessivas deficiências tal como sugere Vargas *et al*. (2013) e há ainda um longo caminho a percorrer no que respeita à modelação num cenário de alteração de

clima (Lawrence *et al.*, 2007; Misson *et al.*, 2007). Neste estudo de Vargas *et al*. (2013) compararam-se estimativas do balanço de carbono e de água ao nível do ecossistema utilizando modelos de base processual, com medições efectuadas em carvalhos de folha caduca e persistente na região mediterrânica utilizando o método do fluxo turbulento. Concluiu-se que os modelos subestimaram de forma significativa e generalizada a fotossíntese durante a estação seca. A capacidade fotossintética é muito superior aquela que os modelos estimam, o que é explicado em parte pela ausência de representação da variação sazonal do nível da toalha freática e do acesso à água do subsolo pelas raízes profundas. Isto traduz-se numa mais elevada eficiência de uso de água que não é considerada nos modelos, resultando numa subestimação da fotossíntese real. Outro problema resulta no fracasso da representação da respiração total do ecossistema. Entre outros factores, salientam-se problemas relacionados com a: 1) representação do efluxo de CO_2 dos solos após pulsos de chuva no Verão, 2) variação da contribuição da componente heterotrófica e autotrófica na respiração do solo, 3) ausência de representação do movimento ascencional da água no perfil do solo, por acção das raízes profundas, a partir do subsolo/toalha freática (*hydraulic lift*). Outro factor limitante relaciona-se com a representação de ecossistemas multiestratificados, com herbáceas, arbustos e árvores, como é frequente na região mediterrânica.

2. Alterações recentes do clima em Portugal e cenários futuros

Os registos meteorológicos passados referem que a temperatura média global na superfície terrestre aumentou 0.6±0.2ºC durante o séc. XX e que na Europa este aumento foi de cerca de 0.95ºC (WMO, 2004). A taxa de aumento das temperaturas médias globais era em 2000 de 0.17±0.05ºC por década (IPCC, 2001) mas o novo relatório do IPCC (2007) veio rever em alta estas estimativas, indicando uma taxa de aquecimento secular, entre 1996 e 2005, de 0.74ºC.

Em Portugal, o panorama climático do último século assemelha-se ao observado no resto do mundo. Em relação à temperatura média, registaram-se 2 períodos de aquecimento significativo: de 1910 a 1945 e a partir de 1975 até hoje e, entre eles, um período de arrefecimento moderado (1945-1975). Salienta-se que, no actual período de aquecimento, o aumento da temperatura tem ocorrido à taxa de 0.45ºC/década, correspondente ao dobro da taxa de aumento registada em termos globais (Karl *et al*, 2000, Tomé e Miranda, 2004). Nos últimos 18 anos a temperatura média anual foi sempre superior ao valor médio de 1971-2000 com excepção de 2008. Também na Primavera se observaram alterações relevantes das temperaturas, tendo-se vindo a registar uma tendência de aumento evidente especialmente nos últimos 30 anos, com temperaturas médias acima dos valores observados no período anterior (1971-2000) (Figura 5, em baixo).

Esta subida das temperaturas médias na Primavera tem sido acompanhada por uma diminuição estatisticamente significativa da precipitação para a mesma estação durante as últimas 3 décadas, com ligeiros aumentos para as outras estações. Sobretudo após 2003 a precipitação total anual tem vindo a ser sistematicamente inferior aos valores médios registados no período de 1971-2000 (Figura 5, topo). Esta condição climática na Primavera é particularmente importante para a vegetação por se tratar da altura do ano em que ocorre o pico do crescimento vegetativo.

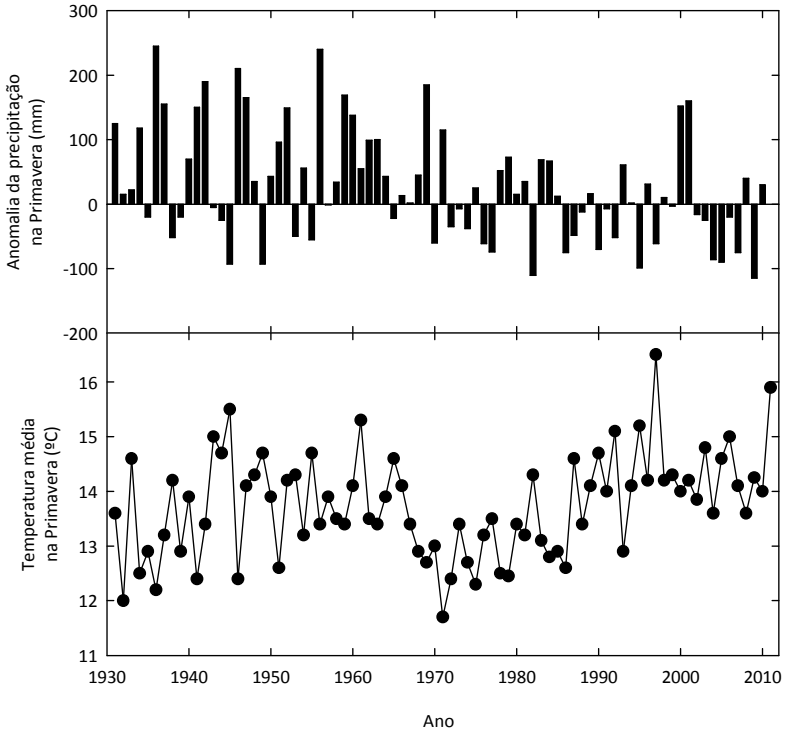

Figura 5 – Desvio da precipitação total anual e evolução das temperaturas médias do ar na Primavera em Portugal continental nos últimos 80 anos em relação à normal 1971-2000 (Fonte: IPMA, 2003 http://www.ipma.pt/pt/).

Ramos *et al.* (2011) efectuou uma análise dos eventos extremos ocorridos em Portugal e na Península Ibérica nos últimos 30 anos e observou um aumento estatisticamente significativo do número de noites tropicais e da frequência de ondas de calor, sobretudo na primavera e verão, e uma diminuição dos extremos de frio no Inverno. Os anos de 2004 e 2005 por exemplo, foram os mais secos desde os últimos 75 anos. Para além do risco de incêndio aumentar grandemente, fenómenos de secas recorrentes poderão debilitar o estado fisiológico das árvores e tornar as plantas mais susceptíveis à acção de outros stresses, de origem climática ou biótica.

Quanto aos cenários de clima futuro para Portugal, os resultados do projecto *SIAM - Climate change in Portugal: Scenarios, Impacts and adaptation measures* referidos no capítulo de Miranda *et al.* (2006) apontaram para um aumento sistemático das temperaturas médias no Verão na ordem dos 3 a 7ºC para o final do século, sobretudo nas regiões do interior do Norte e Centro do país, com um aumento da

intensidade e frequência das ondas de calor. Quanto à precipitação, apesar de maior incerteza dos modelos, estimam-se perdas na ordem dos 20 a 40% com as maiores reduções a ocorrerem no Sul do país e no Inverno. A equipa de trabalho que estudou as florestas, incidiu sobre o estudo da produtividade nas três principais espécies florestais em Portugal Continental: o pinheiro bravo, o eucalipto e o sobreiro. Utilizaram-se dados climáticos diários gerados pelo modelo climático regional do Hadley Centre (HadRM2) para correr o modelo de base processual GOTILWA+, que foi parametrizado para cada espécie para simulação da produtividade actual (simulação de controlo) e uma simulação do futuro (período de 2080 a 2099). Os resultados apontam para um aumento ligeiro da produtividade no Norte Litoral, em consequência da maior humidade e da atenuação da limitação pelas baixas temperaturas. A região Centro é uma região de transição, onde poderá haver pequenos aumentos de produtividade nos locais mais pluviosos, mas a tendência geral será para uma redução da produtividade. Na Região Sul os impactos serão mais severos, verificando-se uma forte diminuição da produtividade, que poderá significar a substituição do coberto florestal por comunidades arbustivas ou mesmo herbáceas anuais nas zonas de maior aridez. Face a estes cenários climáticos, o potencial de sequestro de carbono nas florestas em Portugal tenderá a diminuir no futuro.

3. Importância das florestas nas emissões de gases com efeito de estufa em Portugal

Para além do valor intrínseco das florestas, o serviço ambiental de armazenar e sequestrar o CO_2 da atmosfera é hoje de extrema importância num contexto de alteração climática. O reconhecimento internacional deste serviço ambiental, conceptualizou-se através da assinatura por Portugal de um conjunto de acordos para as quais se submetem anualmente inventários dos GEE e outros poluentes atmosféricos (Protocolo de Quioto, da Convenção Quadro das Nações Unidas sobre as Alterações Climáticas, da Convenção sobre Poluição Atmosférica Transfronteira de Longo Alcance e da Directiva relativa aos Tectos Nacionais de Emissões).

De acordo com o último inventário nacional de GEE de 2010 (Portuguese Environmental Agency, 2012), as florestas e actividades de uso do solo em Portugal representaram um sumidouro líquido de carbono de 9.9 Mt de CO_2 eq. em 2010, compensando em 14% as emissões totais registadas nesse ano de 70.6 Mt CO_2 eq. As florestas contribuíram ainda para que Portugal tenha ficado, nos últimos anos, abaixo dos limites de emissão impostos pelo acordo de partilha de emissões da União Europeia de +27% em relação às emissões de 1990. Em 2010 este valor cifrou-se em +17.5%. Existe ainda um longo caminho a percorrer nomeadamente na optimização da eficiência energética nos vários sectores de actividade.

A importância das florestas enquanto sumidouro de carbono transcende estes compromissos internacionais. A consciencialização por parte da sociedade, de que as alterações climáticas são uma realidade a médio prazo, tem sido materializada na criação de programas de compensação de emissões de gases com efeito de estufa. Entre eles está o mercado voluntário de créditos de carbono. Presentemente, a função de sequestrar carbono é o único serviço ambiental que tem valor de mercado e que pode ser transaccionado. Esta externalidade (positiva) poderá traduzir-se em benefícios económicos no futuro não só para os proprietários florestais, uma vez que muitos deles contemplam programas de florestação, mas também os próprios agentes, pela oportunidade de mostrarem responsabilidade ambiental e diferenciar a oferta dos seus produtos. Porém, no contexto de crise mundial em que vivemos "o mercado de carbono (compra e venda de licenças ou créditos de carbono) está praticamente morto e dificilmente será reanimado sem um acordo mundial de redução de emissões, no qual a União Europeia terá que assumir compromissos de redução que por agora não se encontram na agenda política" (Inês Mourão, Directora da CAOS - Borboletas e Sustentabilidade).

4. Objectivo geral e interesse do estudo

O objectivo desta dissertação foi estudar o balanço de carbono em ecossistemas florestais nos seus principais reservatórios: árvores, arbustos e solo. Focou-se o estudo nos ecossistemas mediterrânicos, em particular os do sul de Portugal. Importantes alterações de uso do solo têm acontecido nestas regiões nas últimas décadas, nomeadamente a declínio do sobreiro, aumento da área de matos e a expansão da área de pinhal manso em povoamentos puros ou em consociação com o sobreiro. Os cenários de alteração de clima apontam no sentido de um aumento do stress ambiental com redução da produtividade e sobrevivência de muitas florestas. Entender os processos de funcionamento destes ecossistemas, no que respeita ao seu balanço de carbono, é fundamental para que a gestão futura das florestas seja consciente e que os riscos de perda das suas funções sejam mínimos.

Assim, o artigo ***Biomass allometry and carbon factors for a Mediterranean pine (Pinus pinea L.) in Portugal*** teve como objectivo específico preencher a lacuna de informação existente em Portugal sobre modelos alométricos de estimação da biomassa para uma das espécies emergentes no actual panorama florestal nacional – o pinheiro manso. Este estudo foi conduzido no âmbito do projecto "O Sequestro de Carbono e Gestão Florestal Sustentável no Sul de Portugal", apoiado pela Acção 8.1 - Desenvolvimento Experimental e Demonstração, do Programa AGRO. Além da construção de modelos alométricos para a biomassa e volume, os dados recolhidos serviram para caracterização de outras variáveis importantes para quantificações de carbono em povoamentos puros de pinheiro manso: densidade básica da madeira, teor de carbono das componentes e factores de expansão da biomassa.

O artigo ***Carbon sink strength of a Mediterranean cork oak understory: how do semi-deciduous and evergreen shrubs face summer drought?*** teve como principal objectivo avaliar a contribuição de um subcoberto arbustivo, composto por espécies semi-caducifólias e perenes, para o sequestro de carbono de um montado de sobro. Este artigo foi desenvolvido no âmbito do projecto internacional *IMECC - Infrastructure for Measurement of the European Carbon Cycle* financiado pelo 6º Quadro comunitário de apoio Europeu. A motivação para este estudo surgiu da oportunidade única de conciliar medições de produtividade líquida do ecossistema pelo método do fluxo turbulento, com medições de fluxos de carbono e água ao nível dos arbustos. Estudos da contribuição do subcoberto para o balanço líquido de carbono ao nível do ecossistema são raros, sobretudo para a região mediterrânica onde este estrato da vegetação surge com frequência nos espaços florestais. Este sítio experimental continua a monitorizar o montado na Herdade da Machoqueira do Grou em Coruche pelo método do fluxo turbulento.

O artigo ***Soil water availability strongly modulates soil CO_2 efflux in different Mediterranean ecosystems: models calibration using the Bayesian approach*** vem reforçar o pressuposto de que a disponibilidade de água dos solos é determinante nas emissões sazonais de CO_2 dos solos mediterrânicos. A motivação para este estudo resultou da oportunidade de reunir medições de respiração do solo, utilizando o mesmo equipamento e o mesmo protocolo de campo, realizadas por várias equipas de investigação do grupo *Forest Ecology and Genetics Research Group* do Instituto Superior de Agronomia (www.isa.utl.pt/cef/ForEcoGen). A base de dados resultante, com mais de 6000 medições pontuais de respiração do solo, permitiu avaliar a sensibilidade de modelos simples de estimação da respiração do solo baseados em dados climáticos, utilizando a estatística Bayesiana.

Esta dissertação explora uma multiplicidade de métodos de quantificação dos reservatórios e fluxos de carbono nos ecossistemas florestais mediterrânicos. Através de uma aproximação multidisciplinar, permitiu clarificar alguns processos biológicos que afectam as respostas das plantas e do solo da região mediterrânica a variações dos factores ambientais culminando com a publicação de artigos científicos e de capítulos em livro. No âmbito deste doutoramento foi ainda produzido o seguinte artigo científico publicado numa revista de circulação nacional: Correia, A. C., Faias, S., Tomé, M., Evangelista, M., Freire, J., Ochoa, P. (2008). Ajustamento simultâneo de equações de biomassa de pinheiro manso no sul de Portugal. Silva Lusitana Vol 16 (2): 197-207.

Esta dissertação está organizada da seguinte forma: é apresentado um enquadramento do estudo através da Introdução no Capítulo 1, seguida da apresentação dos 3 artigos científicos que foram desenvolvidos ao longo do tempo respectivamente nos Capítulos 2, 3 e 4 e uma conclusão geral no Capítulo 5. De referir ainda que na Introdução foram utilizados adaptações de documentos desenvolvidos durante este doutoramento e que são:

- ✓ Pereira, J.S., A.C. Correia, A.V. Correia and J.G. Borges 2009. Florestas. In Ecossistemas e Bem-Estar Humano. Avaliação para Portugal do Millennium Ecosystem Assessment Eds. H.M. Pereira, T. Domingos, L. Vicente and V. Proença. Fundação da Faculdade de Ciências da Universidade de Lisboa, Lisboa, pp. 183-212.

- ✓ Correia, A. C., Pereira, J. S., Mateus, J., Pita, G., Miranda, P., Correia, A. V. In Influência das alterações climáticas na cultura do eucalipto: cenários possíveis in A. A. M. Alves, J. S. Pereira, e J. M. N. Silva, (eds.) Impactes Ambientais do Eucaliptal em Portugal. ISAPress, Lisboa. 2007

Esta tese foi desenvolvida com o apoio de uma bolsa de doutoramento financiada pela Fundação para a Ciência e Tecnologia (REF: SFRH/BD/39058/2007).

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Resumo

As florestas são importantes no balanço global de carbono porque sequestram parte do dióxido de carbono emitido pelas actividades humanas. Como tal, estimativas quantitativamente precisas são necessárias para responder, não só aos compromissos nacionais no âmbito de inventários de gases com efeito de estufa, mas também no âmbito dos mercados de créditos de carbono e como ferramenta de apoio na gestão florestal. Porém, em Portugal carecem estudos para algumas espécies.

O objectivo deste estudo foi desenvolver modelos matemáticos, nomeadamente equações e factores de conversão e expansão que permitem ao gestor florestal estimar os reservatórios de carbono em povoamentos de Pinheiro manso (*Pinus pinea L.*) independentemente da informação de inventário existente sobre o povoamento. Foram abatidas 53 árvores do centro e sul de Portugal representado diferentes classes de diâmetro à altura do peito (6.5 a 56.3 cm), idades (10 a 45 anos) e densidades de povoamento (20 a 580 árv. ha^{-1}). Desenvolveram-se equações de volume e biomassa acima do solo, factores de expansão de biomassa por componente, quantificou-se a densidade básica da madeira e a fracção de carbono nas componentes de biomassa das árvores. Apresenta-se um factor de conversão raiz-parte aérea baseado em árvores cujo sistema radicular foi completamente escavado.

Os resultados indicam que a alometria do pinheiro manso não se compara com a de outros pinheiros semelhantes e varia consideravelmente com as características do povoamento: 1) o factor de expansão da biomassa total acima do solo decresce com o aumento da densidade (de 1.33±0.03 para 1.07±0.01 Mg m^{-3}) devido à alocação da biomassa do tronco para os ramos, b) a densidade básica do lenho média foi 0.5±0.01 Mg m^{-3} mas varia com a dimensão da árvore, c) a razão raiz-parte aérea foi 0.3±0.03, d) o factor de conversão da biomassa em carbono foi estatisticamente diferente do factor comumente utilizado de 0.5. Os resultados sugerem a necessidade de desenvolver factores e equações específicos para a espécie e o local onde serão aplicadas.

As equações e factores de conversão apresentados permitem quantificar o reservatório de carbono em povoamentos de pinheiro manso em Portugal contribuindo para uma quantificação mais rigorosa do balanço de carbono nestes ecossistemas.

Abreviaturas

BEF_i	Biomass expansion factor (Mg m^{-3}) in each componente (i)
c	circumference at breast height (m)
cr	crown radius (m)
d	stem diameter at breast height (cm)
G	stand basal área (m^2 ha^{-1})
h	tree height (m)
h/d	slenderness index
hc	height of the crown base (m)
Hdom	dominant height (m)
LAI	leaf area index (m^2 m^{-2})
M_{bias}	model bias
$M_{efficiency}$	model efficiency
$M_{precision}$	model precision
N	stand density (trees ha^{-1})
pp	annual precipitation (mm)
Root-to-shoot	fraction of belowground to aboveground biomass
rpi	press residuals
T	air temperature (ºC)
V	stem volume (m^3 ha^{-1})
WBD	Wood basic density (Mg m^{-3})

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems

Forest Systems 2010 19(3), 418-433
ISSN: 1131-7965
eISSN: 2171-9845

Biomass allometry and carbon factors for a Mediterranean pine (*Pinus pinea* L.) in Portugal

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Abstract

Forests play an important role in the global carbon balance because they offset a large portion of the carbon dioxide emitted through human activities. Accurate estimates are necessary for national reporting of greenhouse gas inventories, carbon credit trading and forest carbon management but in Portugal reliable and accessible forest carbon measurement methodologies are still lacking for some species. The objective of this study was to provide forest managers with a comprehensive database of carbon factors and equations that allows estimating stand-level carbon stocks in *Pinus pinea L.* (*P. pinea)*, regardless of the tree inventory information available. We produced aboveground biomass and stem volume equations, biomass expansion factors (BEF) by component as well as wood basic density (WBD) and component carbon fraction in biomass. A root-to-shoot ratio is also presented using data from trees in which the root system was completely excavated. We harvested 53 trees in centre and south Portugal covering different sizes (6.5 to 56.3 cm), ages (10 to 45 years) and stand densities (20 to 580 trees ha^{-1}). The results indicate that aboveground allometry in *P. pinea* is not comparable with other pines and varies considerably with stand characteristics, highlighting the need to develop stand-dependent factors and equations for local or regional carbon calculations. $BEF_{aboveground}$ decreases from open (1.33 ± 0.03 Mg m^{-3}) to closed stands (1.07 ± 0.01 Mg m^{-3}) due to a change in biomass allocation pattern from stem to branches. Average WBD was 0.50 ± 0.01 Mg m^{-3} but varies with tree dimensions and the root-to-shoot ratio found was 0.30 ± 0.03. The carbon fraction was statistically different from the commonly used 0.5 factor for some biomass components. The equations and factors produced allow evaluating carbon stocks in *P. pinea* stands in Portugal, contributing to a more accurate estimation of carbon sequestered by this forest type.

Key words: *Pinus pinea; c*arbon balance; climate change; biomass inventory.

Resumen

Alometría de la biomasa y factores de carbono para un pino Mediterráneo (*Pinus pinea* L.) en Portugal

Los bosques juegan un papel importante en el balance global del carbono porque desplazan una gran porción del dióxido de carbono emitidos por actividades humanas. Se necesitan estimaciones precisas para los informes nacionales de los inventarios de los gases de efecto invernadero, mercados de créditos de carbono, y manejo de carbono en los bosques. Pero en Portugal todavía faltan, para algunas especies, metodologías de mediciones fiables y accesibles de carbono en los bosques. El objetivo de este estudio es proporcionar a los gestores forestales una base de datos completa de los factores y ecuaciones del carbono que permitan estimar los stocks de carbono a nivel de rodal, en *Pinus pinea,* independientemente de la información disponible de los inventarios de árbol. Producimos ecuaciones de biomasa del matorral, y del volumen del troco, factores de expansión de biomasas (BEF) por componente así como densidad básica de la madera (WBD) y fracción de los componentes de carbono en la biomasa. Un ratio raíz-tallo se presenta también utilizando datos de los arboles en los que los sistemas radicales se extrajeron completamente. Se cosecharon 53 árboles en el centro y sur de Portugal, cubriendo tamaños diferentes (6,5 a 56,3 cm), edades (10 a 45 años), y densidad del rodal (20 a 580 árboles ha^{-1}). Los resultados indican que la alometria del sistema radical en *P. pinea* no es comparable con otros pinos y varía considerablemente con las características del rodal destacando la necesidad de desarrollar factores dependientes del rodal y ecuaciones para cálculos de carbono a nivel local o regional. $BEF_{radical}$ disminuye de rodales abiertos ($1{,}33 \pm 0{,}03$ Mg m^{-3}) a cerrados ($1{,}07 \pm 0{,}01$ Mg m^{-3}) debido a cambios en la asignación de biomasa de tronco a ramas. La media de WBD es $0{,}50 \pm 0{,}01$ Mg m^{-3} pero varía con la dimensión de los

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Received: 15-04-10; Accepted: 22-09-10.

árboles y el ratio de raíz a tallo se encuentra entre 0,30 ± 0,03. La fracción de carbono fue estadísticamente diferente del factor comúnmente utilizado de 0,5 de algunos componentes de biomasa. Las ecuaciones y los factores producidos permiten la evaluación de los stocks de carbono en rodales de *P. pinea* en Portugal, contribuyendo a una información más precisa del carbono secuestrado por este tipo de bosque.

Palabras clave: *Pinus pinea;* balance de carbono; cambio climático; inventario de biomasa.

Introduction

Reliable estimates of forest carbon stocks and changes over time are necessary to understand the global carbon cycle and to know to what extent they contribute to mitigate greenhouse gas emissions. Feasible and comprehensive carbon estimates in forests are important under international commitments as the Kyoto Protocol or the United Nations Framework convention on Climate Change but also because there is a growing interest of enterprises and landowners in getting involved in the market opportunities available for forest carbon offset credits (Hamilton *et al.*, 2007). The quantification, reporting and verification of carbon sequestered by forests are frequently not as transparent as it should and this has major implications on policy decisions regarding forest conservation and management (Clark *et al.*, 2001).

Methods to assess carbon stock at stand level

Inventory-based methods are the most common for assessing forest carbon stock and changes. IPCC (2003) suggests that this can be done either by directly applying allometric models that predict tree biomass components based on field measurements of individual trees (like diameter at 1.30 m or tree height), or using multiplication factors that allow to convert or expand stem volume to the tree biomass component wanted. This involves the development of site-specific allometric equations requiring tree harvesting and weighting in the field, which is expensive and time-consuming. The use of existing equations comes as an alternative but potential intersite variation in wood quality and biomass allocation throughout stand maturity may introduce errors in the final estimates (Lehtonen *et al.*, 2004). Biomass expansion factors (BEF) and/or conversion factors (CF) are the most widely used methods for biomass calculations because they readily convert, in only one step, stand volume estimates (usually available in forest inventories) in biomass or carbon. Both are multiplication factors but BEF allows to expand growing stock volume to whole or merchantable biomass components of the stand (*e.g.* crown) while CF includes wood basic density (WBD) and carbon fraction conversions. Multiplication factors, foreseen by IPCC Good Practice Guidelines in the case where no biomass information is available, can be species-specific or represent broad species groups (see review in Somogyi *et al.*, 2006). As is the case for generic allometric equations, they do not account for intersite variability and the matching to the site under study requires previous measurements and tree harvesting. According to IPCC (2003), the uncertainty on using generic BEF, WBD and root-to-shoot ratios is considered to be about 30% but Ravindranath and Ostwald (2008), for example, state that the use of generic factors may produce misleading estimates with errors as large as 70%. In spite of the growing number on allometric relationships and expansion factors, ecosystem specific studies continue to be important because they help to reduce this uncertainty.

Pinus pinea overview

In this paper we studied an emblematic Mediterranean species – pinion or umbrella pine (*Pinus pinea* L.), a native pine of Southern Europe covering about 650,000 ha of the Mediterranean Basin (Quézel and Medáil, 2003). The major interest of this species in Portugal is the production of edible nuts, and for that stands must be managed in a way to reach a maximum of 100 trees ha^{-1} at the mature phase which occurs around 30 years old. Even with a large inter-annual cone production variability, which depends on the climatic conditions during the 4 years cone development period, *P. pinea* provides higher incomes to owners because pine seeds are highly prized in the international markets, much more than other forest resources (*e.g.* timber) (Mutke *et al.*, 2005b). Other distinctive characteristic of the species is its genetic uniformity (Vendramin *et al.*, 2008) combined with a high level of fenotipic plasticity (Mutke *et al.*, 2005a). In fact *P. pinea* appears either in arid inland or coastal sea areas affected by salinity stress, and can potentially help mitigating

desertification problems in these particular areas. Drought tolerant species from Mediterranean regions like *P. pinea* may be interesting in a climate change scenario because they are naturally adapted to warmer climates and to water stress. Future scenarios of vegetation distribution predict that these species may shift towards higher latitudes due to climate change (Benito Garzon *et al.*, 2008; Ohlemuller *et al.*, 2006) and turn out to be potentially interesting in future afforestation/reforestation programs in regions becoming susceptible to droughts. *P. pinea* stands managed in long rotations and with moderate wood productivity may also sequester large carbon stocks and contribute to mitigate greenhouse gas emissions. A carbon sequestration simulation made by Del Rio *et al.* (2008) using a integrated single tree model (PINEA2) in even and uneven aged *P. pinea* stands over a 100-year period, estimated 1.2 to 1.5 Mg of carbon sequestered per ha and per year (MgC ha^{-1} $year^{-1}$). These values are underestimated since carbon accumulated in the soil was not accounted, only the annual tree growth and cone production.

The main objective of this paper is to provide the tools to help stakeholders in quantifying carbon stocks for *P. pinea* at stand level, regardless of the inventory information available for the site, and that can either be used for national quantifications, local studies as an alternative to the existing factors that revealed inappropriate. Biomass and volume allometric equations, biomass expansion factors, biomass and carbon conversion factors where developed for the species using destructive tree sampling.

Material and methods

Stand description, climate and vegetation

P. pinea in Portugal covers 3% of total forest area (83,900 ha) but the area had grown 10% in the last 10 years (Tomé *et al.*, 2007). Although occurring throughout the country, most of the area is located in the south where climate and soil allows higher commercial cone yield. The 17 stands used for the selection of the trees for destructive sampling are located in center and south Portugal (Fig. 1) covering most of the *P. pinea* distribution in Portugal. The climate is Mediterranean with a hot and dry summer, with a mean annual temperature of 16°C. Average annual precipitation (1961-1990) varies slightly between stands ranging from 572 to 735 mm with more than 80% occurring between October and April.

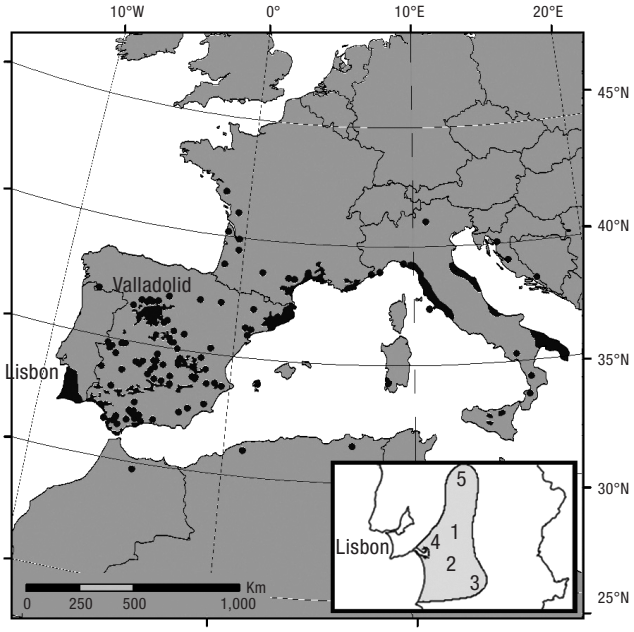

Figure 1. *Pinus pinea* distribution in Europe in 2008. Picture adapted from Fady *et al.* (2004). The box in the right represents the location of the stands used for biomass harvesting in Portugal (in correspondence with the numbering from Table 1); the light gray represents the geographical area of the 101 pure plots inventoried.

Stands are pure, except in one site (Pegões) in which *P. pinea* is mixed with cork oak or maritime pine. The understory consists of grazed pastures with annual C3 herbaceous plants and, in some stands, dispersed shrubs of *Ulex* and *Cistus*. Soil types ranged from sand, loamy sand and sandy loam textures, derived from sand stones sedimentary rocks. All of them are nutrient poor. The stand density ranged from 20 to 580 trees ha^{-1} and LAI ranged from 1.5 to 11, calculated as the sum of all side needle area in the plot (m^2) to the plot area (m^2) (Table 1).

Sample tree selection

A total of 53 *P. pinea* trees were harvested for biomass, stem volume, wood basic density and carbon in biomass analysis. The sampling was stratified by 5 cm diameter at breast height from 5 to 60 cm. Stem diameter at breast height (1.30 m) (d), tree height (h), height to the crown base (hc), crown radius (cr) were measured in each tree. Stand variables were subsequently calculated, namely stand density (N), stand basal area (G) and dominant height (hdom) (Table 1).

Table 1. Characteristics of the sites used for harvesting

Location	Lat(N)/ Long(W)	T (°C)	pp (mm)	Plots, trees W, trees V	Stand characteristics (min-max)					Trees harvested (min-max)			
					LAI (m^2 m^{-2})	hdom (m)	N (trees ha^{-1})	G (m^2 ha^{-1})	Stand age	d (cm)	h (m)	hc (m)	cw (m)
(1) Pegões	38°37'67" -8°37'36"	15.9	709	6, 15, 33	3.3-5.3	13.9-18.1	60-207	10.1-15.3	40	8.0-56.3	3.7-17.3	2.2-10.0	1.4-5.1
(2) Palma	38°28'07" -8°38'40"	16	572	2, 5, 5	4.1, 4.2	14.6, 18.1	110, 130	20.1, 20.7	45	29.4-43.2	11.0-12.0	2.2-4.3	4.1-5.4
(3) Ferreira do Alentejo	38°08'29" -8°18'18"	16.2	586	2, 4, 4	2.5, 2.8	10.9, 15.0	74, 170	7.7, 8.4	40	9.2-30.9	2.7-10.1	0.9-6.2	1.3-6.8
(4) Gâmbia	38°33'17" -8°47'30"	16.1	735	1, 10, 5	11	6.6	580	14.6	13	7.5-20.4	2.6-5.9	0.9-1.9	1.1-2.2
(5) Coruche	38°57'34" -8°25'45"	16	642	6, 6, 6 including roots	1.5-4.9	5.3-11.4	20-120	1.5-7.5	20	6.5-46.0	2.1-7.7	0-1.6	1.2-4.7

Climate: T, mean annual temperature (°C); pp, total annual precipitation (mm). Stand characteristics: Plots, number of plots inventoried in the stand; trees W, number of trees harvested for biomass; trees V, number of trees used for volume; LAI, leaf area index (m^2 m^{-2}); hdom, stand dominant height (m); N, stand density (trees ha-1); G, stand basal area (m^2 ha^{-1}); Stand ag, of the stands. Characteristics of the trees harvested: d, diameter at breast height (cm); h, tree height (m); hc, height to the crown base (m); cw, crown radius (m). Minimum and maximum values separated by dashes.

Aboveground biomass sampling

The main aboveground biomass components —stem wood, stem bark, branches and needles— were weight in 40 trees by harvesting and additionally other 13 where measured for volume calculations. Due to limited economic resources, the root biomass was weight by the excavation method in 6 of them. In some trees the cones had already been harvested (the harvesting period in Portugal is between 15th December and 31st March) so cones were not used in the models. In fact, due to the importance of cone production, there are specific models already available to predict cone weight in the country (Freire, 2009). Trees were cut from the stump, and dead and living branches were separated from the stem. The stem wood identification inside the crown was sometimes difficult, since trees tend to produce bifurcations due to the lack of apical dominance. In these cases, the most vigorous branch was selected to represent this stem fraction. The stem was then fractioned in 2 m width logs (in small trees every 1 m) and weighted in the field. A disk was collected from the base of each log and weighted in the field and kept for laboratory determination of dry weight and for stem wood/bark proportion calculations. The cross diameters of the disks with and without bark were measured in fresh conditions for volume calculations. A systematic sampling approach was used to collect at least 20% of the crown biomass choosing the 1st branch in each group of 5 starting from the base to the tip of the crown, for wood and needle separation. The remaining crown was weight in the field but without any component separation. Branches were divided in 3 diameter classes: less than 2.5 cm, between 2.5 and 7 cm and more than 7 cm and these fractions weight. Portable digital scales with 1 g precision were used for field weightings. For each tree component a sample of approximately 200-500 g was kept and sealed in labeled plastic bags for laboratory determination of dry weight. The biomass of each component was calculated by the product of dry weight to fresh weight ratio of the component sample and the total fresh weight of the component in the tree, estimated by the proportion of the component in the total fresh weight (Porté *et al.*, 2002; Ritson and Sochacki, 2003).

For tree volume we summed the volume calculated for each intermediate log with the Smalian formula assuming a cylinder geometric shape for the stump and intermediate logs and a cone geometric shape for the top log. On average, the separation and weighting of each tree took 2 hours with the work of 5 persons but the time spent in aboveground biomass separation varied according to the tree dimensions: the maximum was 5 hours with 7 persons for a tree with 3.5 Mg (fresh weight) and the minimum was 1 hour with 5 persons for a tree with 0.035 Mg (fresh weight).

Root sampling

Six trees were used for root biomass determination using the excavation method. The trees were selected from stands with different soil types within the same area (Regosol, Cambisol and Luvisol) (FAO, 1998) and with different, yet low, stand densities (Table 1). After cutting the tree from the stump, a geometric polygon was identified on the ground taking into account crown projection and/or half the distance to neighborhood trees. The stump and the soil within the marked area were removed with a hydraulic excavator, and roots were separated in piles according to soil horizons and depths. The maximum depth excavated varied between 1 and 2 m according to the depth of the sandstone rock and the root system development of the tree. In each pile, roots were manually separated, washed to remove soil particles and exposed to open air to dry. The same procedure was used for the stump. Roots were separated in 3 diameter classes: less than 5 mm (fine), between 5 and 30 mm (small) and more than 30 mm (coarse), and then weighted in the field. Portable digital scales with 1 g precision were used for field weightings. For each tree component, two samples were collected and sealed in labeled plastic bags for dry weight and carbon analysis. All the samples were oven-dried to constant weight at 70°C for dry weight calculations. The total dry biomass for each component was estimated from fresh weight based on sample dry weights.

Biomass and volume equations

The allometric relationship between tree biomass (or volume) and tree or stand variables usually takes the form of a power function and has been widely studied (see review in Zianis *et al*, 2005). In this study we tested the following power functions: $w = k_i x^a$ and $w = k_i x^a y^b$, where w is the dependent variable that can be either biomass or volume, where k, a and b are parameters to be estimated by regression analysis and x and y are tree variables. The tree variables tested were: d (stem diameter at 1.3 m), h (tree height), hc (height to the crown base), cr (crown radius) and the slenderness index (h/d).

Sapwood area was also tested as a candidate explanatory variable. For sapwood area calculation, the surface of the disk measured at the base of the living crown was sanded on one side and then scanned on a flatbed scanner. Sapwood area and the number of rings in each disk were measured in 4 perpendicular directions using the software WindENDRO (Regent Instruments, Sainte-Foy, Quebec, Canada). Sapwood area was only used to predict needle biomass in an independent model (following the methodology described above), since only 32 out of the 40 trees sampled for biomass had this measured done. These equations were built because the relationship between needle biomass (and area) to sapwood area is closely linked with plant productivity (Ryu *et al.*, 2010) and is a common variable in carbon balance models based in the pipe-model theory (Waring *et al.*, 1982). This theory proposes that a given unit of leaf (biomass) area is supplied with water from a constant quantity of conducting pipes and can help explain some biomass patterns in *P. pinea* trees with stand development.

For each component (volume with bark, stem bark, stem wood, branches and needles), the non-linear models were fitted by the least squares method, using all possible combinations of variables. The root biomass was not included in the simultaneous fit because only 6 trees were used. The performance of the biomass components models was evaluated based on the Press residuals (r_p) (Myers, 1990) guaranteeing the significance of all the parameters in the selected models. The normality of the model errors was evaluated by plotting the studentize residuals over the normal residuals (QQ-plots). The non-normality was overcome in the final model with iteratively reweighted least squares regression using the Huber function to reduce the influence of data containing large errors (Myers, 1990). To overcome heterocedasticity, the models were fitted with weighted non-linear regression, using Parresol (1999) methodology to calculate the weights. Models were evaluated using the methodology proposed by Soares *et al.* (1995) and Vanclay and Skovsgaard (1997), which consists of calculating the following statistics based on the PRESS residuals (r_{pi}):

1) Model efficiency ($M_{efficiency}$) computed as:

$$M_{efficiency} = 1 - \frac{\sum_{i=1}^{n} r_{pi}^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2}$$

where r_{pi} is the Press residual from tree *i* (residual obtained when tree *i* is not included in the fit) for the dependent variable, $\bar{y}$ is the average value and *n* is the number of trees measured;

2) model bias (M_{bias}), expressed by the r_p average;

3) model precision ($M_{precision}$) expressed by the average of the absolute values of r_p. The best models

were the ones with higher efficiency and lower bias and precision values. The models selected for each tree biomass component (except the root component), including total tree aboveground biomass expressed as the sum of the biomass components, were simultaneously fitted with the non-linear seemingly unrelated regression (Parresol, 1999; 2001). Model fittings where performed with SAS software (SAS, 2004).

The aboveground biomass measured in the 40 trees harvested was correlated with the estimates obtained with a set of biomass equations from Spanish *P. pinea* stands (Fig. 1) developed by Montero *et al.* (2005) and the results are presented in Table 3. These consist of 7 biomass equations (for total aboveground, stem, branches of less than 2 cm, between 2 and 7 cm and higher than 7 cm, needles and roots) adjusted with allometric functions using d as the independent variable. The data set used 47 trees harvested in the region of Huelva, Valladolid (Spain) (Fig. 1) with d ranging from 7.5 cm to 63 cm. No information was provided regarding stand characteristics. The average temperature (12°C) and the annual precipitation (435 mm) (1971-2000) is lower than the sites analyzed in this study probably as a result of the continental influence.

Expansion factors - biomass aboveground and root-to-shoot ratio

To compute BEF_i we used a total of 101 pure *P. pinea* permanent plots selected from a national grid (Freire, 2009) that used the same inventory methodology described in section 2.2 (Table 2). No trees were harvested from these plots, these were only used to compute BEF_i. The subscript index (*i*) refers to needle, branches, stem wood, stem bark, stem (wood + bark), crown (branches + needles) or total aboveground biomass, these last 3 thereafter named aggregated BEF. BEF_i is computed as follows:

$$BEF_i \ (\text{Mg m}^{-3}) = \frac{\text{Biomass}_i \ (\text{Mg ha}^{-1})}{\text{Volume with bark}_i \ (\text{m}^3 \ \text{ha}^{-1})}$$

Volume with bark and biomass where estimated using equations from Table 3 (presented in the *Results* section). BEF_i values where plotted with stand variables (N, hdom and G) in order to study possible trends in biomass allocation patterns.

The root-to-shoot ratio refers to the roots and stump biomass (Mg) over total aboveground biomass components (branches, needles, stem wood and stem bark) (Mg), and was calculated for the 6 trees in which root biomass was measured.

Conversion factors - Wood basic density (WBD) and Carbon fraction in biomass

WBD is defined here as the ratio of wood biomass (oven-dry matter) (Mg) to the green volume (m^3) and was measured in wood disks (without bark) taken each 2 m (or 1 m long) from the stem. The knots and resin wounds were separated in the disks in order to avoid local data overestimation. The displacement of volume by immersing the wood in water was the method used (Zobel and Buijtenen, 1989). Wood and bark were oven-dried until a constant weight at 60-80°C for dry weight calculations. Tree WBD was calculated by averaging WBD in each disk weighed by the corresponding diameter without bark. The variation of WBD between and within trees was first assessed by graphical analysis. Trees were grouped in 3 height classes (trees from 2 to 6 m, 6 to 10 m and higher than 10 m) and the average WBD (and the corresponding standard error) was calculated at each intermediate height (at the base, 2 m, 4 m, 6 m and so on). A regression analysis was then performed, using tree d, h and discs height level within the tree, as well as interactions between these variables, as regressors in order to test the significance of the effect of tree size and height level on WBD.

Table 2. Main characteristics of the 101 *Pinus pinea* plots inventoried and used for BEF_i calculations and aboveground biomass calculations

	N (trees ha^{-1})	hdom (m)	G (m^2 ha^{-1})	V (m^3 ha^{-1})	Aboveground biomass (Mg ha^{-1})
Min	18	4	2	6	7
Mean	194	12	10	53	63
Max	1647	21	26	175	194

Table 3. Final models selected for biomass components (in kg) and volume with bark (in m^2) considering tree variables as predictors

	Equation	Average (min-max)	Par.	Estimates	R^2 ajd	M_{bias}	$M_{precision}$	$M_{efficiency}$
Needles	$k\ c^a\ (h/d)^b$	38 (3-222)	k	22.27 (18.09)	0.71	2.68	11.56	0.63
			a	1.76 (1.69)	(0.83)	(0.97)	(5.05)	(0.82)
			b	–0.50 (–0.67)				
Branches	$k\ c^a$	194 (1-1,182)	k	184.94 (23.56)	0.79	1.03	66.00	0.74
			a	3.03 (1.84)	(0.79)	(0.85)	(5.89)	(0.76)
Stem bark	$k\ c^a\ h^b$	21 (1-78)	k	8.08 (6.85)	0.83	0.83	4.99	0.82
			a	1.55 (1.46)	(0.83)	(0.97)	(5.05)	(0.82)
			b	0.47 (0.54)				
Stem wood	$k\ c^a\ h^b$	154 (5-675)	k	18.85 (19.26)	0.94	2.03	26.27	0.93
			a	1.68 (1.61)	(0.95)	(2.19)	(26.31)	(0.94)
			b	0.95 (0.94)				
Total aboveground	wl + wbr + + wb + ww ($k\ c^a\ h^b$)	480 (20-1,899)	k	280.1	0.92	6.57	77.27	0.91
			a	2.33	(0.91)	(10.45)	(74.04)	(0.91)
			b	0.19				
Volume with bark	$k\ h^a\ d^b$	0.66 (0.02-2.73)	k	0.000094				
			a	0.65		0.0028	0.00865	0.93
			b	1.97				

d: diameter at breast height (cm). c: circumference at breast height (πd/100) (cm). h: tree height (m). Average (min-max) represents the average, minimum and maximum biomass values in each biomass component used to build the models. For biomass models, the values represent the parameters and several model fitting validation tests after simultaneous fitting (in kg). In bracket the validation tests before simultaneous fitting.

In order to take into account the clustered structure of the data, with correlation among consecutive observations within the same tree, model error was modeled as an autoregressive process. The effect of tree size on the tree average WBD was also assessed with regression analysis.

A total of 70 biomass samples were collected for carbon concentration analysis. The purpose was to collect randomly 10 samples of each biomass components (needles, branches, stem bark, stem wood and roots) from different trees. However the number of samples varied due to several reasons. For roots we only used 6 trees. For needles we add 8 samples from a study carried out in the department on *P. pinea. F*or branches we used the 10 samples collected in each of the 3 branch diameter classes described in section 2.3. For stem bark we used 6 samples because 4 out of 10 lead to inconsistent results. The method used was the dry combustion method, according to ISO Standard 10694, using a CNS elemental analyzer. To test whether the average values were significantly different from the commonly used 0.5 carbon fraction, we looked at the confidence interval (with 95% confidence) for each biomass component.

Stand carbon stock comparisons

From a carbon budget management perspective we may question which stands hold de maximum carbon stocks: mature stands with a few trees or young stands with high tree density? In an attempt to answer this question for the *P. pinea* stands in this study, we plotted stand basal area measured (G, $m^2\ ha^{-1}$) for the 101 stands inventoried, with the total carbon in the trees estimated in each stand divided in 5 stand density classes. The estimates for aboveground biomass in each stand were calculated by applying the biomass equations from Table 3 (to each tree), converted in carbon using the carbon fraction for each component (from Table 5) and expressed by unit area (ha).

Results

Allometric relationships

The total aboveground biomass of sample trees varied between 20 and 1,899 kg, the branch and needle biomass ranging from 1 to 1,182 kg and from 3 to 222 kg,

respectively. The stem wood ranged from 5 to 675 kg and the stem bark from 1 to 78 kg (Table 3). The branches represented the most important fraction of the aboveground tree biomass (average 43% ± 2%) followed by the stem wood (average 37% ± 2%), needles (average 13% ± 1%) and stem bark (average 7% ± 0.3%). Diameter at 1.3 m (d) and height (h) were the most important independent variables in all equations and accounted for more than 71% of the variability of the different biomass components and volume equations (Table 3).

Overall validation statistics with simultaneous fitting were better for branches but decreased substantially for needles remaining practically the same for stem (wood and bark) components (Table 3). The $M_{efficiency}$ for total aboveground biomass was the same for both sets (0.91), but for needle biomass equation in an independent model using sapwood area was high, suggesting that this variable provides a good means to predict needle biomass (Fig. 2).

For volume with bark, the values varied between 0.02 to 2.73 m^2 with a high $M_{efficiency}$ (n = 52).

The biomass estimates using Montero *et al.* (2005) equations are well correlated with the observed values (see Fig. 3). When testing the probability of the differences between variances of the two datasets we conclude that there are statistical differences for needles (F-test; $p = 0.01 < 0.05$) but not for stem and branches ($p = 0.20$ and 0.38). However when looking at the absolute errors

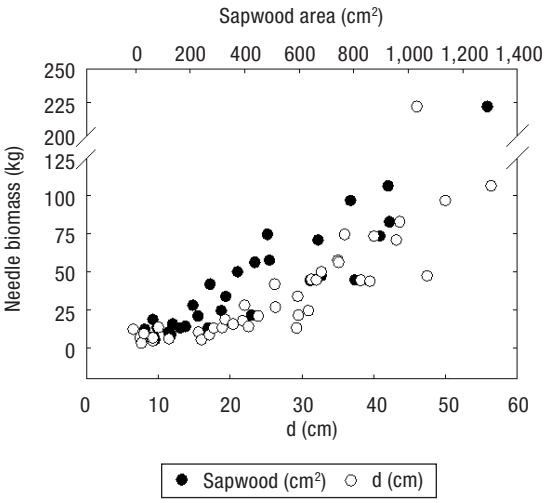

Figure 2. Needle biomass (kg) (y) expressed as a function of (x) that can be either d (cm) or sapwood area (cm^2) measured at the base of the live crown. Nonlinear model fitted: $y = k\ x^a$. For the fitting with: a) x = d: k = 0.06, a = 1.87, $R^2adj = 0.62$, $E_{efficiency} = 0.59$, n = 40; b) x = sapwood: k = = 0.06, a = 1.08, $R^2adj = 0.78$, $E_{efficiency} = 0.70$, n = 32.

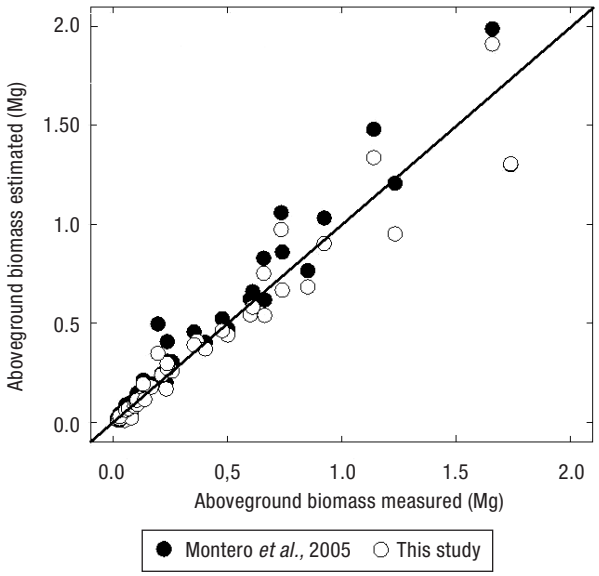

Figure 3. Left graph: correlation between the total aboveground biomass (Mg) measured in the 40 trees harvested and the estimates obtained using Table 3 equations and the biomass equations for *P. pinea* developed by Montero *et al.* (2005) (in Mg).

(difference between the measured and the estimated value) we observed that the estimates are quite biased. The average percentage error computed as the average of the relative error (difference between the measured and the estimated value over the measured value expressed as a percentage) for the 40 trees, resulted in an overestimation for stem and branches (22.2% and 55.3%, respectively) and a underestimation for needles of 23.4% using Montero *et al.* (2005) equations.

Needles/aboveground biomass tends to decrease with stand development (from 21% ± 3% at < 20 cm d class to 9% ± 3% at d > 40 cm) as well as the stem/aboveground biomass with a minimum at d > 0 cm with 36% ± 5% (Fig. 4c). For the ratio of branches/aboveground biomass there is a tendency to increase as stands gets less denser reaching a maximum at d > 40 cm with 55% ± 4%. For trees less than 20 cm (that is young trees in dense stands), biomass allocation to the crown (and specially branches) seems to be a priority, representing more than 60% of the total aboveground biomass.

The observed decrease in the ratio of productive (needle) to respiring biomass (branches+stem wood + stem bark) as stand gets older (Fig. 4a and 4b) is rather expected (Ryan and Yoder, 1997) and is related with higher construction and maintenance respiration costs with woody tissues. Nevertheless, this decreasing rate is very smooth for d classes higher than 20 cm (average

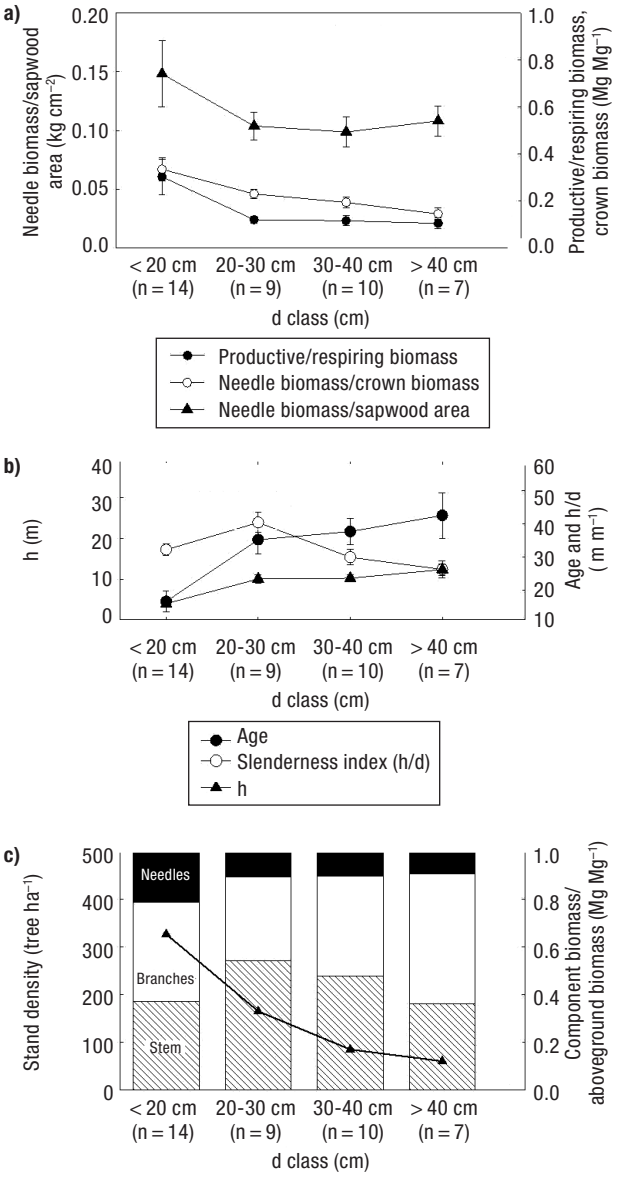

Figure 4. Average values and standard errors calculate for each diameter at breast height class for the following variables: a) Ratio between the productive (needle) to respiring biomass (branches + stem wood + stem bark) (Mg Mg^{-1}); ratio between the needle biomass to crown biomass (Mg Mg^{-1}); ratio between the needle biomass to sapwood area measured at the crown base (kg cm^{-2}). b) h: height (m); age; h/d: height/diameter at 1.3 m (m m^{-1}). c) Stand density (trees ha^{-1}); ratio between each biomass component to total aboveground biomass: Black boxes (needles), white boxes (branches), stripe boxes (stem). n in the axis represent the number of trees used in each class.

12% ± 0.3%). The slenderness index was low at d < 20 cm because trees at this stand density levels invest little in height compared with diameter (Fig. 4b) and presented a peak at 20-30 cm decreasing thereafter.

Expansion factors and root-to-shoot ratio

Average BEF_i values were higher for branches with 0.57 ± 0.015 Mg m^{-3} followed by stem wood (0.42 ± 0.002 Mg m^{-3}), needles (0.12 ± 0.005 Mg m^{-3}) and stem bark (0.06 ± 0.002 Mg m^{-3}) (Table 4). The aggregated BEFwas higher for total aboveground (1.18 ± 0.01 Mg m^{-3}), followed by the crown (0.69 ± 0.014 Mg m^{-3}) and then for stem (wood + bark) (0.49 ± 0.003 Mg m^{-3}). The highest variability was found for $BEF_{branches}$.

Plotting the aggregated BEF with stand variables (N, G, hdom and Volume), N was the variable that presented the best correlations with BEF_i (Fig. 5) showing a decreasing pattern for aboveground and BEF_{crown} and an increase for BEF_{stem}.

Maximum BEF_{crown} was 1.08, decreasing to 0.41 in very dense stands with a typical exponential decay curve shape when plotted over stand density (Fig. 5 and Table 4). The opposite is observed for BEF_{stem} with a much smaller variation from 0.41 to 0.57. $BEF_{aboveground}$ showed the same tendency than BEF_{crown}..

Regarding the root system, we observed that roots between 5 and 30 mm represents the higher fraction of the total root biomass (63%), followed by fine (20%) and coarse roots (17%). The root-to-shoot ratio found for the 6 trees harvested was 0.30 ± 0.03. The horizontal superficial root exploration observed in the surface layer exceeded 1.5 to 2 times the tree crown projection area and about 90% of the total root biomass was in the top 50 cm. Regarding root development in depth, we also observed that fine roots were able to penetrate in severely compacted horizons which can be extremely important for groundwater uptake in the dry season.

Table 4. Biomass expansion factors (BEFi in Mg m^{-3}) for each tree component (needles, branches, stem bark and stem wood) and aggregated BEFi (in Mg m^{-3}) (crown, stem and total aboveground). Columns represent the average, minimum, maximum and the standard error (s.e.) for BEFi for each component. The number of plots used for BEFi calculations equals 101 (stand characteristics are described in Table 2)

BEF_i	Average	Min	Max	s.e.
Needles	0.12	0.06	0.28	0.005
Branches	0.57	0.28	0.99	0.015
Stem bark	0.06	0.04	0.11	0.002
Stem wood	0.42	0.36	0.11	0.002
Crown	0.69	0.41	1.08	0.014
Stem (wood + bark)	0.49	0.41	0.57	0.003
Total aboveground	1.18	0.97	1.50	0.010

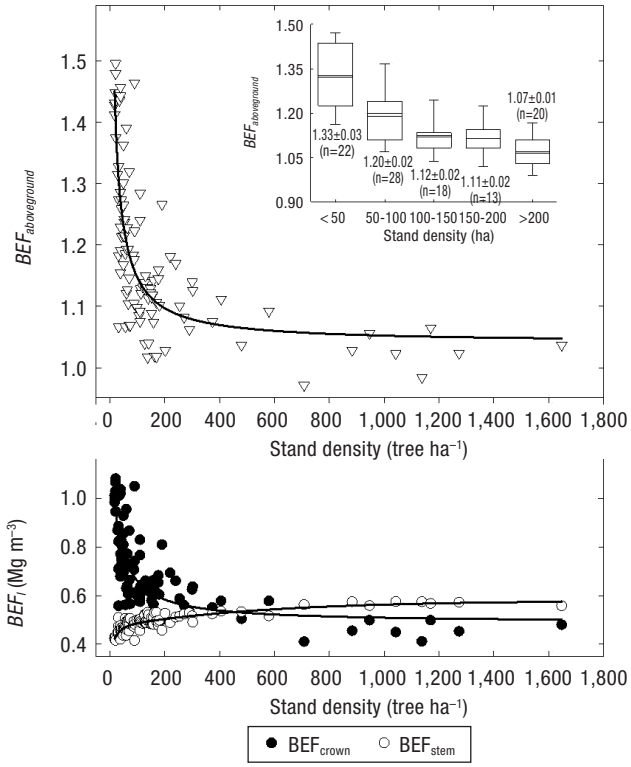

Figure 5. Biomass expansion factors (BEF_i, Mg m^{-3}) plotted with stand density. Graph in the bottom: BEF_{crown} (branches and needle biomass over volume with bark) and BEF_{stem} (wood and bark biomass over volume with bark). Graph in the top: $BEF_{aboveground}$ (aboveground biomass over volume with bark). Biomass in Mg ha^{-1} and volume in m^3 ha^{-1}. Lines represent the trend line (n = 101). Right graph: box plot of $BEF_{aboveground}$, by stand density classes. Average and median values represented in the box with a strong and smooth line, respectively. Box boundaries are 25 and 75th percentiles and 90th and 10th percentiles in whiskers. Average values, standard error and the number of plots used in each density class represented in the top of each box.

Conversion factors

Wood basic density

Average WBD for all trees weighted by the disks cross diameter in the stem was 0.51 ± 0.01 Mg m^{-3} (min-0.43 Mg m^{-3} and max-0.60 Mg m^{-3}). Figure 6 shows the vertical distribution of WBD in trees with different sizes. Taller trees presented the higher average values (0.55 ± 0.01 Mg m^{-3}) and the shorter trees showed the lowest (0.47 ± 0.02 Mg m^{-3}). We also observed higher values in the base of the stem compared to the top. The regression analysis lead to the following model with the model error modeled as a 2nd order autoregressive process (all parameters significantly different from zero for p = 0.05):

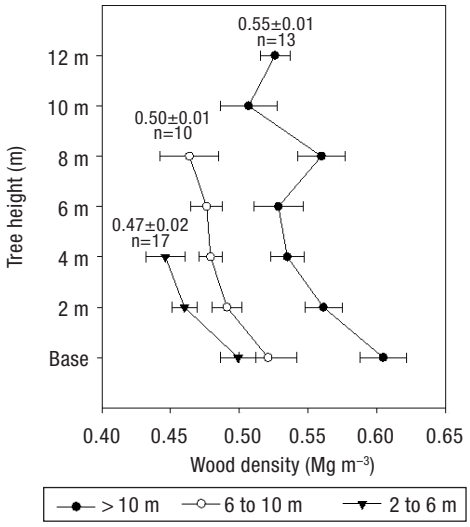

Figure 6. Mean wood basic density (Mg m^{-3}) in each height classes (2 to 6 m, 6 to 10 m and > 10 m). Standard errors represented in horizontal bars. Values in the top are the average, standard error and number of samples used for calculations in each high class. All pairwise multiple comparison procedures (no statistical differences for 2 to 6m and 6-10 m class, p = 0.226, Tukey test).

$$WBD = 443.63 + 10.17\ h\text{-}20.78\ h_i + 20\ h\ h_i \quad (R^2 = 0.46),$$

where WBD is wood basic density (Mg m^{-3}), h is tree total height (m) and h_i is height level within the tree (m). Tree diameter at breast height, alone or combined with tree height, did not improve the model for WBD. The following model was found for tree average WBD:

$$WBD = 437.51 + 8.03\ h \quad (R^2 = 0.50),$$

where the symbols are as before. These analyses clearly show that WBD depends on tree height, increasing with tree size. Within the tree, WBD decreases from the soil level to the tip.

Carbon fraction in biomass

Average carbon fraction in the dry biomass for branches and roots is not significantly different from 0.5 carbon fraction (Table 5), but they are for stem wood, stem bark and needles taking into account a 95% confidence interval.

Stand carbon stock comparisons

Taking into account the above methodologies, we observe in Figure 7 that the maximum carbon stock in the aboveground biomass is reached in open stands,

Table 5. Carbon fraction in biomass components (Mg of carbon per Mg of dry matter). Columns represent the average, minimum, maximum, the standard error and the number of samples used for each component. Conf. int. (95%) represents the 95% confidence interval for the population mean

Carbon fraction	Average	Min	Max	s.e.	n	Conf. int. (95%)
Needles	0.45	0.42	0.46	0.003	18	0.44-0.45
Branches	0.51	0.49	0.55	0.002	30	0.50-0.51
Stem bark	0.54	0.53	0.56	0.005	6	0.53-0.55
Stem wood	0.53	0.50	0.59	0.008	10	0.51-0.55
Roots	0.50	0.50	0.51	0.002	6	0.50-0.51

which correspond to trees in the mature and reproductive stage. Stand carbon stocks are successively higher in stands with less trees compared with dense (and younger) stands for the same G. This will allow to identify, from a carbon budget perspective, which is the more compensatory strategy: protect mature or old stands with large biomass stocks from harvesting and degradation, or to plant new trees that will take a couple of decades to get big enough to have the same amount of carbon as the mature ones. The aboveground estimates are within the range of values reported in the literature for other pines. For example, Janssens *et al.* (1999) found an average of 103 tC ha^{-1} in a 69-year-old *Pinus sylvestris* in a Belgian Campine region with 556 tree ha^{-1}. Grunzweig *et al.* (2007) refers an average value of 177 tC ha^{-1} in a 35 year-old with 300 trees ha^{-1} in Negev Desert, Israel. Evrendilek *et al.* (2006) studied several conifers in Turkey and reported an average of 137 tC ha^{-1} in a *Pinus nigra* of 92 year-old forest with 300 trees ha^{-1}. King *et al.* (2007) reported an average of 115 tC ha^{-1} in a 55 year-old *Pinus resinosa* with 622 trees ha^{-1} forest in the Upper Peninsula of Michigan.

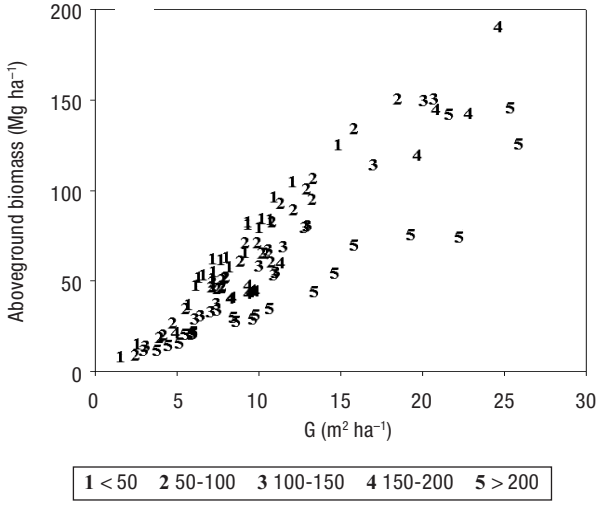

Figure 7. Total aboveground biomass in *P. pinea* stands (Mg C ha^{-1}) plotted with stand basal area (G, m^2 ha^{-1}) in 5 stand density classes: (1) < 50 tree ha^{-1}, (2) 50-100 tree ha^{-1}, (3) 100-150 tree ha^{-1}, (4) 150-200 tree ha^{-1}, (5) > 200 tree ha^{-1} in the 101 inventoried (Table 2).

Discussion

Biomass equations

The tree variables d and h had a significant effect on the allometric relationships for biomass and volume, in agreement with other authors (António *et al.*, 2007; Calama and Montero, 2006). Some studies had tested other variables with success, namely with tree age for eucalypts in Congo (Saint-Andre *et al.*, 2005), stand age for *Pinus pinaster* in Aquitaine (Porté *et al.*, 2002), sapwood area to stem diameter for several boreal species (Bond-Lamberty *et al.*, 2002). The inclusion of the slenderness index (ratio h/d) as a predictor in the needles biomass for *P. pinea* model is possibly related with the response of needle biomass, more than other tree components, to competition since the h/d index is usually associated with tree social status in the stand (Ilomaki *et al.*, 2003).

Simplified biomass models dependent on commonly measured variables like d and h, are advantageous from the application point of view, either for field inventories or data processing. In this study we fitted the equations for the tree component simultaneously with the total biomass expressed as the sum of the components. This was made in order to take into account the correlation between the errors of the several models and also to guarantee that the total biomass estimation (by summing the estimates for each independent biomass component equation), is equal to the total aboveground estimated by the aboveground biomass equation. This restriction has not been taken into account by several authors (*e.g.* Saint-Andre *et al.*, 2005) with the consequent inconsistence in total biomass estimates.

Comparison wiht independent biomass equations

The differences found between the biomass measured and estimated by Montero *et al.* (2005) may be explained by the fact that these equations only use d as a explanatory variable. On the other hand, the fact that trees from Montero *et al.* (2005) study come from a more continental climate may cause the proportional lower needle biomass (Fig. 1). Concomitantly Montero *et al.* (2005) found, on average, a higher proportion of branch biomass in relation to the aboveground tree biomass (51% compared with 43% in this study) which can be explained by stand management. However no information is available regarding this issue. These differences in biomass estimates highlights the importance of validating equations and factors outside the range of ecological region from where they are going to be applied. However, the number of trees sampled and the small geographical area from which the trees were harvested in this study is clearly very limited for making any generalizations in the statistical sense. Nonetheless, the results provided here can be useful as a general indicator of overall relationships in *P. pinea* in the most important Portuguese productive area.

The equation for needles after simultaneous fitting was notably worse than the independent model (Table 3). Since the average needle biomass in total aboveground biomass is not neglectful (around 10%), the set of biomass equations after simultaneous fitting should be used when all the components have to be quantified together. However, for isolated estimates of this component (for example leaf area calculations), the independent needle equation from Table 3 should be used. Alternatively, we propose 2 independent equations (Fig. 2) based on d and sapwood in a power function that can come as alternatives for use in some carbon balance models, since the independent variables are closely linked with physiological processes underlying these models.

Stand development and biomass partitioning

Needles

From Figure 4c we conclude that younger *P. pinea* trees ($d < 20$ cm) allocate considerably more resources to the production of photosynthetic tissue ($21\% \pm 0.03\%$) compared to the remaining aboveground tree component. The higher investment in the needle biomass is a common survival strategy in the juvenile phases but the proportion in relation to aboveground tree biomass decreases as stands get older due to the formation of new wood structures and the maintenance of the older ones (Ryan and Yoder, 1997). The decrease in the proportion of productive (needles biomass) to respiring biomass (stem wood, stem bark and branches) reflects this trend (Fig. 4a): the decrease is abrupt in the first stages (from $30\% \pm 8\%$ in < 20 cm d class to $12\% \pm 2\%$ in 20-30 d class) but then slows down beyond this class. This is consistent with the observations for the ratio needle biomass to sapwood area: a significant decrease in the ratio of needle biomass to sapwood area from $d < 20$ cm to 20-30 cm class (0.15 ± 0.03 kg cm^{-2} to 0.10 ± 0.002 kg cm^{-2}) and then maintenance above 30 cm d class in around 0.10 kg cm^{-2}. This means that trees continue to produce leaves as a response to light availability with no limitations in water supply to the crown, since the sapwood area increases concomitantly (McDowell *et al.*, 2002; Mencuccini and Grace, 1995).

Stem

The lower h/d index found at $d < 20$ cm at high densities (average 326 tree ha^{-1}) is not expected, mainly because the h/d in the following d class is higher (Fig. 4b). Although some authors refer that trees in competition tend to invest more in height growth instead of diameter growth (Ilomaki *et al.*, 2003), this does not seem the case in this study. It is possible that for these small trees, stand density is not enough to stimulate height growth as seem to be the case in the above mentioned study (tree density ranged from 400 to 5,000 tree ha^{-1}, respectively). On the other hand, the proportionally high needle biomass found at these stages, consistent with *P. pinea* light demanding characteristic, suggest a higher tree investment in a more efficient water conducting system and therefore in d (and sapwood) growth. The decrease in the slenderness index after 20-30 cm d class is a result of the light exposure due to the stand density decline, suggesting the higher investment in diameter growth with a concomitant stabilization of height growth at 11 m above 20-30 cm d class (Fig. 4b). This is advantageous from the hydraulic point of view since the hydraulic resistance increases as trees get older and taller, resulting in limits to carbon assimilation (Ryan and Yoder, 1997). On the contrary, *P. pinea* trees seem to be investing in main-

taining the balance between needle biomass to sapwood area (Fig. 4a) probably as a strategy to protect the xylem water conducting system. This is rather important at the peak of reproductive phase, when all the resources are being allocated to fruit production (around 30 years old). Many authors working with other species (McDowell *et al.*, 2002; Mencuccini and Grace, 1995; Waring *et al.*, 1982) refer that the ratio of needle area/sapwood area must decrease with increasing h and age, in order to reduce hydraulic resistance. This is especially important in dry climates where vapor pressure deficits are higher. This has been reported in several species from temperate to dry climates (DeLucia *et al.*, 2000), but have never been studied for *P. pinea*. In this study, this was evident in the first stages of the life of the tree (from d class < 20 cm to 20-30 cm) but stopped thereafter, even with an increase in age and the (smooth) increase in h. Above 20-30 cm d class, no structural adjustments were recorded regarding needle biomass/sapwood area. This could either be explained by the low intra-specific competition mentioned above or to the ability of the root system to support foliage with water, which seems to be efficient in *P. pinea*, in open stands (see results for roots in section 3.2). This fact was also observed for Holm oak in South Portugal (David *et al.*, 2007).

Branches

It is noteworthy that the proportion of branches gradually increases as stand density declines reaching more than 40% of the aboveground biomass (Fig. 4c). This has probably to do with a *P. pinea* strategy to enhance seed production at reproductive stages (Mutke *et al.*, 2005b). Note that if there were any hydraulic limitations, the first structures to be damaged would be the tip of the branches where the hydraulic resistance is higher (Mutke *et al.*, 2003). However at these stages, the absence (or reduced) competition for light, and probably the efficient access to water, allow co-dominant branches to stiffen due to secondary growth and to be maintained in the canopy surface in the best light conditions, suffering less down-bending. These heavier and stronger branches are the ones able to sustain more and heavier cones (Mutke *et al.*, 2005c). This explains why higher cone productions can only be achieved in open stands (usually with less than 100 trees ha^{-1}).

This higher allocation pattern to branches in the mature stands may also explain why older stands with less trees are able to retain more carbon than denser stands with the same G (Fig. 7). This will allow to identify, from a carbon budget perspective, which is the more compensatory strategy: protect mature or old stands with large biomass stocks from harvesting and degradation, or to plant new trees that will take a couple of decades to get big enough to have the same amount of carbon as the mature ones. Concomitantly, Mutke *et al.* (2005b) emphasizes that the biomass allocation to cone production is similar to stem volume growth which may exacerbate the importance of open and mature stands to overall carbon stock and sequestration.

Expansion factors and root-to-shoot ratio

Average BEF_i values found in this study (Table 4) are amongst the highest reported for Mediterranean and Northern European species and much higher than other pines (Gracia and Sabaté, 2002; Lehtonen *et al.*, 2004; Levy *et al.*, 2004; Peichl and Arain, 2007). The higher $BEF_{aboveground}$ found is in consistence with the species structure and geometry – lack of apical dominance with a polyarchic ramification resulting in a crown shape wider than deeper (Mutke *et al.*, 2005c) and with a proportionally heavier crown compared with the rest of the aboveground woody tissues. Biomass allocation pattern for *P. pinea* changes significantly with stand density (Fig. 5) so stand density is, to a certain extent, a surrogate for stand development, with older stands having the lower densities. This conclusion shows that the use of a single average value for the species or species group for biomass calculations at stand level in *P. pinea* can lead to highly biased estimates. This has been well demonstrated by several studies on other species (Lehtonen *et al.*, 2004; Tobin and Nieuwenhuis, 2007).

Regarding the root-to-shoot ratio, there is still a huge lack of publications on consistent and comparable methods of root measurements in adult trees and very few on *P. pinea*. The error associated with fine roots biomass sampling was considered to be very small using this methodology because all the soil volume explored by the roots was taken into account including root development in depth till the sandstone rock (Carlos Pacheco, personal communication). Janssens *et al.* (1999) for example also states that roots with less that 1 mm represents less than of the total tree biomass. Fernández (2004) reported a root-to-shoot ratio of 0.19 for *P. pinea* in Andalusia, 0.24 for *Pinus stobus* in Canada (Peichl and Arain, 2007) and 0.26 in the review

of Cairns *et al.* (1997). Levy *et al.* (2004) reported an average root-to-shoot ratio of 0.36 for conifers in Great Britain. IPCC (2003) suggest as default root-to-shoot for conifer forest plantations of 0.32 ± 0.08. A study carried out with *Pinus pinaster* in center Portugal in a Regosol using the same methodology as this study, reported a root-to-shoot of 0.28 ± 0.05 ($n = 5$) (Sónia Faias, personal communication). The 0.3 factor for root-to-shoot found here, although not statistically representative should be used as an alternative to the ratios found in literature. But this is a subject that clearly needs more investigation.

Conversion factors

Wood basic density

Average stem WBD found in this study was 0.51 ± 0.01 Mg dm m^{-3}. Other values in the literature for the same species reports 0.52 ± 0.08 Mg dm m^{-3} for the stem (Gracia and Sabaté, 2002), Oliveras (2003) reported 0.38 ± 0.02 Mg dm m^{-3} for roots and 0.55 ± 0.03 Mg dm m^{-3} for branches. Montero *et al.*(2005) cite the value 0.50 for *P. pinea* although do not mention to which component refers to. Carvalho (1996) reported 0.55 Mg dm $_{12\% \text{ humidity}}$ m^{-3}. The average values reported in this study are therefore very close to the ones referred by other authors for the same species. However there are intra-specific discrepancies that should be taken into account when dealing with very old or very young even-aged stand carbon calculations, justifying in some cases the use of dimension-dependent WBD values. Higher WBD is generally found in taller trees compared with smaller ones which may be related with tree age. The analysis from tree-rings in this study shows that taller trees are also the oldest. This has to do also with a decrease in the juvenile wood and an increase of the heartwood percentage (which has higher WBD) which is frequently accompanied by resin and other extractives. Resinous deposits may be inflating overall WBD in older trees and may also explain the elevated WBD in the base disks since the wounds for resin extraction were performed around breast height. The discrepancy found for WBD inside the crown of the taller trees may be explained by the proximity to foliage, where physical and chemical properties of the juvenile wood are more variable. These results are in consistency with the results reported in Zobel and Buijtenen (1989).

Carbon concentration in biomass

Recent publications strongly discourage the use of the common 0.5 carbon fraction of biomass (Mg of carbon per Mg of dry matter). Bert and Danjon (2006), studying *Pinus pinaster* stands in France, found statistical differences from the 0.5 value for all the tree components carbon concentration, always higher than 0.5. Lamlom and Savidge (2003) found a higher carbon concentration in early wood compared with late wood in conifers, and also argue that the method and sample preparation influence the final carbon concentration results. However, other authors such as Ritson and Sochacki (2003) did not find any statistical difference between carbon concentration in aboveground components in *Pinus pinaster* in Australia and the 0.5 factor. Although we had found a statistical difference between the carbon concentration and the 0.5 factor for some components, we believe that the arguments presented above and the fact that elemental composition of completely dry wood is remarkably uniform (molecular formula $CH_{1.44}O_{0.66}$), the 0.5 factor overcomes the other potential sources of error related with species, stands or the method used in the carbon elemental analysis.

Conclusion

In this study we present and emphasize the importance of species-specific methods for stand level carbon estimates in *P. pinea* stands. We found that biomass allometry varies considerably with stand management, meaning that generic and averaged expansion and carbon factors used to convert volume in biomass can produce misleading carbon estimates. Biomass equations should be species and geographically specific or at least validated and adjusted if necessary to the study area. *P. pinea* stands are important carbon stocks, especially mature stands in the reproductive stages. Maximum carbon accumulated in the trees in mature stands with very low tree densities is comparable to carbon stocks in denser stands from other pines reported in the international literature. This results from the high *P. pinea* investment in crown biomass, especially branches, in order to support a high cone production. Future studies should address the role of *P. pinea* stands as carbon sinks and clearly more investigation should be done over the importance of soil, cones and root systems in overall *P. pinea* carbon balance.

Acknowledgements

The authors wish to thank AFLOPS – Associação de produtores florestais for their help in the field measurements specially Mafalda Evangelista, Marta Bastos and Luis Unas. We also thank Filipe Costa e Silva and Peter Fay for the English review. The first author was supported by a PhD scholarship funded by Fundação da Faculdade de Ciências (SFRH/BD/39058/2007). We also thank AGRO 8.1 - Desenvolvimento Experimental e Demonstração (DE&D) under the project nr 543 for financing this work and project 451 for making available information regarding some inventory plots.

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Povoamento de pinheiro manso na Herdade de Monte Novo de Palma (Concelho de Alcácer do Sal) com 50 anos e 140 árvores por ha

Povoamento em linha de pinheiro manso na Herdade da Gâmbia (Concelho de Setúbal) com 10 anos e 580 árvores por ha

Povoamento de pinheiro manso na Herdade do Outeiro da Mina (Concelho de Ferreira do Alentejo) com 100 árvores por ha


Pormenor da escavação de uma raiz de pinheiro manso com 25 anos (esquerda) e selecção de ramos para separação das componentes (direita)


Pormenor de separação das agulhas dos ramos e pesagem de ramos de acordo com o seu diâmetro


Pesagem e separação de amostras em laboratório

Resumo

Os arbustos mediterrânicos são espécies extraordinariamente bem adaptados às condições xerófitas do nosso clima e de regeneração face ao fogo, possuindo inúmeras vantagens competitivas de uso de recursos limitantes. Os objectivos deste trabalho foram: estudar as estratégias de utilização da água, luz e carbono de arbustos semi-caducifólios e perenes do sub-coberto de um montado de sobro da região mediterrânica durante o Verão e transição para o Outono, avaliar o impacte de um pulso de chuva no Verão nos fluxos de carbono e água ao nível da planta e quantificar a contribuição dos arbustos para o sequestro de carbono ao nível do ecossistema durante este período.

Utilizaram-se câmaras de medição dinâmica de fluxos de CO_2 e água nas 3 espécies dominantes: *Cistus salviifolius*, *Cistus crispus* (semi-caducifólias) e *Ulex airensis* (perenifólia), complementados com medições fisiológicas e morfológicas nas plantas. Um modelo hiperbólico de resposta à luz limitado pelo deficit de pressão de vapor foi utilizado para estimar a fotossíntese dos arbustos por dia e comparado com medições de produtividade primária bruta do ecossistema estimados pelo método do fluxo turbulento.

O deficit hídrico de Verão resultou numa diminuição da área foliar nas espécies semi-caducifólias assim como da fotossíntese em todas as espécies. Todavia, a transpiração e a respiração das plantas variou de forma significativa entre espécies. Embora a espécie de raízes superficiais (*Cistus salviifolius*) tenha utilizado a luz de forma mais eficiente, demonstrou um fraco controlo estomático na transpiração, apresentando taxas de respiração sucessivamente mais altas. O *Ulex airensis* manteve maiores potenciais de água no Verão mas menores taxas fotossintéticas e menor transpiração com a progressão do Verão. Após uma chuvada de 22mm no Verão, o *Cistus salviifolius* usou a água imediatamente disponível de forma oportunista, com hidratação foliar e recuperação da fotossíntese. A espécie de raízes profundantes (*Ulex airensis*) não respondeu ao humedecimento do solo. O prolongamento da seca após a chuvada originou um aumento exacerbado da transpiração e dos custos de respiração nas espécies de raízes superficiais, traduzindo-se numa menor capacidade de recuperar após as chuvas de outono. Conclui-se que uma estratégia oportunista de uso da água poderá não ser vantajosa num cenário climático futuro de aumento da estiagem, associado a uma maior probabilidade de ocorrência de chuvadas.

A contribuição dos arbustos do subcoberto para a assimilação total do ecossistema foi de 17% no Verão/Outono sugerindo que a gestão da densidade da vegetação do subcoberto deve ser considerada numa perspectiva de balanço de carbono.

Abreviaturas

%H	Air relative humidity (%)
ψ_{pd}	Leaf xylem water potential (Mpa)
ABG	Abovegroung biomass (g)
CUE	Carbon use efficiency
DBH	Diameter at breast hight (cm)
ES	Early summer
Fa	Net plant assimilation rate (µmol CO_2 m^{-2} leaf area s^{-1})
Fet	Transpiration (mmol H_2O leaf area m^{-2} s^{-1})
Fnee	Net exchange flux in the light chamber (µmol CO_2 m^{-2} s^{-1})
Fr	Plant respiration (µmol CO_2 g ABG^{-1} s^{-1})
Freco	Soil and plant respiration in the dark chamber (µmol CO_2 m^{-2} s^{-1})
Fsoil evaporation	soil evaporation (mmol H_2O m^{-2} s^{-1})
Fsoil	Soil respiration in the base soil plot (µmol CO_2 m^{-2} s^{-1})
GPP	Gross primary productivity (gC m^2 day^{-1})
LA	Leaf area (m^2)
LAI	Leaf area index
LUE	Light use efficiency (µmol CO_2µmol^{-1} photon)
MA	Mid summer
PAR	Incident photosynthetically active radiation (µmol photon m^{-2} s^{-1})
PARuc	Photosynthetically active radiation undercanopy (µmol photon m^{-2} s^{-1})
pp	Precipitation (mm)
Q	Photosynthetically active radiation (µmol photon m^{-2} s^{-1})
SE	Summer end
SLA	Specific leaf area (m^2 kg^{-1})
SWC	Soil water contente (%)
Tair	Air temperature (ºC)
Tsoil	Soil temperature (ºC)
VPD	Vapor pressure deficit (hPa)
VPDuc	Vapor pressure defici undercanopy (hPa)
WUE	Water use efficiency (µmol CO_2mmol^{-1} H_2O)
α	Initial slope of the light response curve
β	Maximum Fa rate

Journal of Vegetation Science ■■ (2013)

Carbon sink strength of a Mediterranean cork oak understorey: how do semi-deciduous and evergreen shrubs face summer drought?

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Keywords
Cistus sp; CO_2; Eddy-covariance; H_2O; Mediterranean; partitioning; Shrubs; *Ulex* sp; up-scaling

Nomenclature
APG III (2009)

Received 29 October 2012
Accepted 21 May 2013
Co-ordinating Editor: Francisco Pugnaire

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Abstract

Questions: How do semi-deciduous and evergreen shrubs exploit environmental resources during summer drought? What is the contribution of the understorey shrubby layer to ecosystem carbon assimilation? To what extent are carbon balance and transpiration impacted by a rain pulse?

Location: Cork oak open woodland in the Mediterranean region.

Methods: We used closed dynamic light and dark chambers to measure gas exchange (CO_2 and H_2O) in the dominant shrub understorey species *Cistus salviifolius*, *Cistus crispus* (semi-deciduous) and *Ulex airensis* (evergreen), together with plant physiological and morphological measurements during summer drought and autumn recovery. A hyperbolic light response model constrained by vapour pressure deficits was fitted for up-scaling shrub photosynthesis to the ecosystem level. The data were compared, on a daily and daytime basis, with gross primary productivity estimates from ecosystem eddy-covariance flux measurements.

Results: The onset of summer drought led to a significant leaf area reduction in semi-deciduous species. A general decrease in photosynthesis in all species was observed, while evapotranspiration and above-ground respiration fluxes contrasted among species during summer progression and autumn recovery. The shallow-rooted *C. salviifolius* was able to use light more efficiently than the other two species, although with poor stomatal control over water loss and consistently higher above-ground respiration rates, leading to lower water and carbon use efficiencies when compared with *C. crispus*. The deep-rooted shrub *U. airensis* maintained higher leaf water potentials and very low photosynthetic rates while decreasing transpiration rates throughout the summer drought. A summer rain pulse showed that shallow-rooted shrubs use water in an opportunistic way, with immediate leaf rehydration and concomitant photosynthesis increments. Conversely, deep-rooted shrubs (*U. airensis*) were unresponsive, only recovering photosynthesis with high soil water content. An opportunistic growth response may be disadvantageous to shallow-rooted shrubs in a future climate with extended dry summers and higher probability of rain pulse events. The prominent increase in transpiration rates and plant respiration costs observed during the dry conditions that followed the rain pulse, led to a reduced plant ability to recover after autumn rains.

Conclusions: The shrubs that naturaly colonized this montado understorey showed contrasting strategies to overcome summer drought, suggesting an efficient mosaic exploitation of seasonal environmental resources. The contribution of these shrubs to total ecosystem CO_2 uptake during summer and autumn recovery was 17%. This high contribution implies that shrub density management decisions should consider a carbon balance perspective.

Doi: 10.1111/jvs.12102

Introduction

As a transition area between subtropical and mid-latitudes climates, the Mediterranean Basin constitutes both a refuge area and one that encourages floral exchange and active plant speciation (Medail & Quezel 1999). It is one of the world's major centres for plant diversity but also a climate change 'hotspot' within the next century (Lionello et al. 2012).

The territory, which was once covered by evergreen oak forests, deciduous and conifer forests, has undergone profound changes in the past centuries as a result of deforestation, intensive grazing and fires. This has led to major changes in plant community structure and the extension of woodlands dominated by competitive species (Blondel & Aronson 1995). The high species richness recorded in Mediterranean Basin ecosystems is a consequence of certain levels of land perturbation without which biodiversity maintenance would be ecologically unsustainable (Scarascia-Mugnozza et al. 2000). The Portuguese *montado* agroforestry system is such an example. This unique landscape has remarkably high biodiversity, including many rare and endangered species, resulting from both the climatic influence and centuries of human presence (Bugalho et al. 2011). Generalized cork oak morbidity and mortality has been occurring in the last decades throughout southern European countries, attributed in part to recent climatic changes (Aronson et al. 2009). Land abandonment in the traditional cork oak–grassland mosaic has led to progressive shrub encroachment. Highly competitive and opportunistic shrubs are becoming dominant in the understorey of abandoned *montados*. Without adequate silvicultural control, these areas are prone to severe and recurrent wildfires (Acacio et al. 2009). However, there is evidence that allowing a certain level of shrub recruitment is not necessarily detrimental and can in fact enhance biodiversity in Mediterranean forests (Torras et al. 2009). In very depleted sites it can enhance the quantity and distribution of soil and plant carbon and nitrogen pools and increase net ecosystem productivity (McKinley & Blair 2008; Maestre et al. 2009).

The understorey of this cork oak woodland was naturally colonized by highly competitive and successful Mediterranean sclerophyll shrubs, which evolved to either escape or tolerate summer drought stress. Evergreen shrubs (e.g. *Ulex* sp.) are considered tolerant as they maintain a decreasing but positive carbon balance throughout the drought period. On the other hand, semi-deciduous shrubs (e.g. *Cistus* sp.) are drought avoiders, dropping some of their leaves during summer and showing higher productivities only when environmental conditions are favourable (Harley et al. 1987; Werner et al. 1999). The different strategies used to exploit environmental resources may contribute significantly to net ecosystem carbon productivity. How much they really contribute is still a research gap for the Mediterranean region.

Evidence of such a high understorey contribution to ecosystem carbon balance is presented in Misson et al. (2007), in a study dealing with the partitioning of forest carbon fluxes between the overstorey and understorey in ten FLUXNET sites. In general, these studies have shown that the contribution of understorey gross primary productivity (GPP) in summer can reach 39% of total daytime ecosystem GPP. This study used eddy-covariance flux measurements for determining both understorey and overstorey carbon fluxes. At present, this is the most consensual method for disentangling temporal changes in ecosystem carbon fluxes (Baldocchi 2003). Nonetheless, such partitioning studies are sporadic, especially in Mediterranean ecosystems, and there is still much debate regarding methodological issues with the use of the eddy-covariance technique under forest canopies. An example of such problems is the build up of a strong inversion layer in the understorey of open forests, leading to inconsistent nighttime fluxes. In closed forests, deflection of canopy wind direction leads to a discrepancy in overstorey and understorey flux footprints (Misson et al. 2007). This means that complementary measurements in patches of relatively small plants or uniform areas of the understorey are needed. This is especially important in open forest sites, because both understorey leaf area index and light penetration through the canopy are higher, leading to potentially increased understorey biodiversity and GPP. A more detailed description of the understorey also has the advantage of allowing the comparison of instantaneous fluxes from plants and soil with environmental factors (Kolari et al. 2006) and with replicates in space and time. This information can be incorporated in up-scaling modelling exercises and then compared with overstorey eddy-covariance flux measurements.

Without complementary measurements in the understorey, eddy-covariance flux towers do not provide *per se* a detailed understanding of certain processes occurring in the heterogeneous forest understorey to disentangle responses triggered by soils or vegetation. This is the case when summer rain pulses occur. This is a rewetting event in previously dry soils that causes a rapid and transient increase in soil carbon efflux (Tang et al. 2005; Cheng et al. 2006; Jarvis et al. 2007; Unger et al. 2012). However, the living above-ground biomass component also plays an important role, with species responding differently (Cheng et al. 2006; Chen et al. 2009; Patrick et al. 2009). This means that changes in the timing and magnitude of precipitation may have consequences not also for soil dynamics but for the

Doi: 10.1111/jvs.12102

plant carbon balance, with the potential to alter community composition.

In this study, we hypothesize that shrub recruitment can contribute significantly to the cork oak ecosystem carbon sink. Our investigation focused on the contribution of the shrubby understorey GPP to the ecosystem carbon balance of a Mediterranean cork oak woodland. To evaluate carbon flux partitioning between the understorey and the overstorey, we used net ecosystem productivity data from an eddy-covariance flux tower installed above the tree canopies and closed dynamic chambers to measure carbon fluxes in the dominant species of the understorey, as well as efflux from the soil. Our objectives were to investigate: (1) carbon and water exchange dynamics in the main shrub species and their relative sensitivity to increasing summer drought and recovery after the first autumn rains; (2) the contribution of the understorey to ecosystem carbon assimilation; and (3) shrub species responses to a rain pulse that occurred in the middle of the dry season.

Methods

Site description

The experimental site Herdade da Machoqueira is located in central Portugal (39°08′20.9″ N, 9°19′57.7″ W, 165 m a.s.l.) and is integrated in the EUI3 IMECC network. The climate is mediterranean, with wet and mild winters and dry and hot summers. Average annual precipitation is 608 mm, and mean annual temperature is 15.9 °C (period 1971–2000 from Évora meteorological station). The site is a 50-yr-old evergreen cork oak open woodland (*Quercus suber*) with an understorey covered with 2-yr-old shrubs (see Table 1 for other characteristics). The soil is a cambisol (FAO), with 81% sand, 5% clay and 14% silt, with roots mainly in the upper horizons (ca. 0–40-cm depth) and some sinker roots taking water from deeper soil horizons and subsoil.

Tree biomass per hectare was estimated by measuring all tree diameters and heights in a representative plot of 40-m radius. Tree above-ground biomass components (leaves, trunk and branch) were estimated subsequently using species-specific allometric equations (Simioni et al. 2008). Tree leaf area index (LAI) was calculated using leaf biomass estimated from allometric equations and species-specific leaf area (SLA) factors (Vaz et al. 2011).

Soil samples were taken randomly (three points) in the understorey from 0 to 10-cm depth, together with undisturbed soil samples for soil bulk density calculations. Soil organic carbon concentration was determined by the dry combustion method according to the International Organization for Standardization 10694, using a CNS elemental analyser (Leco CNS-2000, MI, US). Nitrogen concentration was determined by Kjeldahl digestion analysis (Digestion System 40; Kjeltec Auto 1030 Analyser, DEcator, SE). Soil organic carbon content was determined using the methodology referred in IPCC (2003).

Table 1. Site vegetation and soil characteristics. Methods related to biomass sampling are reported later in the text.

Characteristic		Units
Trees		
Tree density	177	Trees·ha^{-1}
Height	7.9	m
DBH	24.7	cm
LAI	1.6	m^2·m^{-2}
Total C stock	33.7	tC·ha^{-1}
Shrubs		
Maximum height	80	cm
Total C stocks		
Ulex airensis	0.156	tC·ha^{-1}
Cistus crispus	0.093	tC·ha^{-1}
Cistus salviifolius	0.548	tC·ha^{-1}
LAI (maximum)	0.38	m^2·m^{-2}
Grasses		
Above-ground C stock	0.32	tC·ha^{-1}
% functional groups	43% forbs 45% gram 12% leg	
Soil		
Litterfall input	1.06	tC·ha^{-1}·yr^{-1}
C stock (up to 60 cm)	62.2	tC·ha^{-1}
C/N ratio	21.2	

LAI, leaf area index; DBH, diameter at breast height.

Ecosystem flux measurements

Standard meteorological data on rainfall (ARG100; Environmental Measurements Ltd., Gateshead, UK), solar radiation (BF2; Delta-T Devices Ltd., Cambridge, UK), air humidity and temperature (CS215; Campbell Scientific, Inc., Logan, UT, US) were collected continuously in 30-min time steps (CR10X; Campbell Scientific).

The fluxes of CO_2, water vapour and energy were continuously measured using an eddy-covariance system installed at the top of a metallic tower (22 m). The system consisted of a 3-D sonic anemometer (R3; Gill Instruments Ltd., Lymington, UK) and a closed-path infrared gas analyser (LI-7000; LI-COR Inc., Lincoln, NE, US) measuring, respectively, the three components of wind velocity and temperature, and the concentration of water vapour and CO_2. Data were continuously acquired on a field laptop with EddyMeas (Meteotools, Jena, DE; Kolle & Rebmann 2007).

Eddy flux data were treated using the eddy-covariance data acquisition and processing software package EddySoft (Meteotools; Kolle & Rebmann 2007). Fluxes were determined on a 30-min basis by block-averaging the 20-Hz

data. Time lags between CO_2 or H_2O signals and vertical wind velocity were determined via cross correlation analysis following Aubinet et al. (2000). The sectorial planar fit method was used for the coordinate rotation of wind vectors (Wilczak et al. 2001). The storage term of CO_2 was calculated according to Greco & Baldocchi (1996) and added to the turbulent CO_2 flux. Final steps of the flux processing procedure included data gap-filling and partitioning of the net CO_2 fluxes into GPP and ecosystem respiration according to Reichstein et al. (2005). Further details on flux data-processing, computation and data quality control are described in Pereira et al. (2007).

Understorey measurements

Soil volumetric water content was measured up to 40-cm depth (2, 10, 20, 30, 40 cm) with dielectric soil moisture sensors in four different places (EC5; Decagon Devices, Inc. Pullman, WA, US). Photosynthetic photon flux density was measured at 0.5 m above the ground with 30 quantum sensors (LI-190SA; LI-COR) randomly placed in the understorey. These measurements were automatically collected with 30-min averages. Litterfall input was estimated with 16 L baskets of 1 m^2 placed in two transects across the site and sampled every 15 d throughout 2011, with separation of leaves, branches, inflorescences and acorns.

Shrubs and soil gas fluxes

Shrub gas fluxes were measured for the three dominant species of the understorey: *Ulex airensis*, *Cistus salviifolius* L. and *Cistus crispus* L. *U. airensis* is an endemic species of the Portuguese flora. It is a perennial evergreen shrub with the shoots and leaves modified into green spines. At maturity, *U. airensis* retains a high proportion of dead biomass. *Cistus salviifolius* and *Cistus crispus* are both in the Cistaceae family. They are perennial shrubs with xerophytic characteristics, surviving the Mediterranean summer drought by losing part of their leaves in order to reduce the transpiring area (semi-deciduous habit).

We randomly selected four plants per species for flux chamber measurements. In addition, two plots were selected with bare soil and two with an herbaceous layer. Closed portable chambers of 40 cm × 40 cm and 54-cm high were used to measure CO_2 and H_2O fluxes in each selected plot. With one transparent chamber (light chamber) we measured net CO_2 exchange (Fnee) and transpiration (Fet), and with an opaque chamber (dark chamber) we measured plant and soil respiration (Freco) in the plot. Both chambers were constructed in our laboratory facilities.

The light chamber was constructed from 3-mm thick plexiglas, with more than 95% light transmittance (Hussain et al. 2009). The dark chamber was constructed of opaque PVC, and covered with reflective aluminium foil. To ensure the chambers were completely airtight during the measurements, they were placed over a collar platform with a basal area of 39.5 cm × 39.5 cm and 10-cm high, which was previously buried in the soil leaving a border of 4 cm above the ground. Both the chambers and the collars were fitted with rubber gaskets at the base to ensure airtight conditions. Air temperatures inside and outside the chambers were continuously monitored in order to match ambient conditions during the flux measurements. Ice packs were used in order to keep temperature within 1 °C of ambient. Fans placed inside the chambers provided constant air circulation. Vapour pressure deficit (VPD) changes in the chambers were limited to 1 hPa during the readings when the chambers were placed on the vegetation. We assumed that such small VPD changes should not affect CO_2 exchange via stomatal effects (Otieno et al. 2009). Light levels above the vegetation were monitored using a PAR quantum light sensor (model QSO-S; Decagon Devices) at the beginning and at the end of each measurement. Measurements were performed with an infrared gas analyser (LI-840A; LI-COR) connected to the chamber. Additional information on the methodology is available in Kolari et al. (2006), Li et al. (2008), Otieno et al. (2009) and Langensiepen et al. (2012).

Measurements were conducted from June to November on a fortnightly time step. Each plot was measured in the early morning, midday and in the afternoon, using the light chamber, immediately followed by the dark chamber. To avoid feedback effects on stomata due to an increase in relative humidity inside the chamber, each measurement was reduced to 3 min, with values being recorded each second. During the measurements, no condensation was observed inside the chambers. The time series collected in each measurement were validated for CO_2 concentration linearity with time. Only time series with correlations above 90% were chosen for the calculation of CO_2 and H_2O flux rates, using the equation and methods described in Wohlfahrt et al. (2005) and Li et al. (2008).

To estimate net plant assimilation rates (Fa), we used the equation: Fa = (Fnee + Freco)/LA, where Fnee is the net CO_2 exchange flux measured in the light chamber, Freco is the soil and plant respiration efflux measured in the dark chamber, and LA is plant leaf area measured in each plot. The units are in $\mu mol\ CO_2 \cdot m^{-2}$ leaf area$\cdot s^{-1}$. Plant respiration (Fr) was calculated with the equation: Fr = (Freco − Fsoil)/ABG, where Fsoil in the CO_2 efflux measured in the bare soil plot and ABG is the above-ground biomass measured in each plot. The units are in $\mu mol\ CO_2 \cdot g^{-1}$ above-ground biomass$\cdot s^{-1}$. Water flux measured with the light chamber was used to determine plant

Doi: 10.1111/jvs.12102

transpiration (Fet) and expressed on a leaf area basis; he units are mmol $H_2O \cdot m^{-2}$ leaf area$\cdot s^{-1}$. Soil evaporation corresponds to the flux measured with the light chamber in the bare soil plots and is expressed in mmol $H_2O \cdot m^{-2}$ soil$\cdot s^{-1}$.

Shrub morphological and physiological measurements

Shrub morphological parameters were measured from plant harvesting at the end of the experiment. The four plants used in the plots for gas flux measurements were harvested and the root system completely extracted from a soil volume of 40 × 40 × 40 cm. These plants were used to determine morphological parameters (height, biomass partition, leaf area and root surface and length). SLA was calculated as the ratio between leaf area and leaf dry mass. Leaves and roots were scanned and leaf area and root parameters (length, diameter, area) were calculated using the WinRhizo software (Regent Instruments Inc., Quebec, Canada). All dry mass values were obtained after 48 h at 65 °C.

For shrub biomass extrapolation to the stand level, we sampled four plots in a total of 120 m^2 in the tower footprint area. All shrubs inside the plots were harvested and separated per species for biomass determination. Leaf to total above-ground biomass and SLA were used for leaf area index (LAI) calculations for each species at the stand level.

Leaf xylem water potential was measured at predawn (Ψ_{pd}) with a Scholander-type pressure chamber (PMS Instruments, Corvallis, OR, US) in four plants per species in the summer drought stress period (DOY 215). Shrub leaf samples were taken periodically during the experiment for chloroplast pigment analysis (chlorophyll *a*, *b* and total) describe in detail in Lichtenthaler (1987).

Water, light and carbon use efficiencies

To quantify the trade-off between the amount of carbon assimilated and the amount of water lost by transpiration and evaporation (ET) we used water use efficiency (WUE), which is the ratio between Fa and Fet. Light use efficiency (LUE) is defined as the efficiency with which plants harvest available light to fix carbon via photosynthesis. It was calculated as the ratio of Fa on a leaf area basis and incident PAR. Carbon use efficiency (CUE) was calculated as the ratio between Fa and Fr, which corresponds to the carbon gain via assimilation per each carbon lost via respiration. Two representative days from early summer (June, DOY 173 and 187), summer end (September/October, DOY 270 and 288) and mid-autumn (November, DOY 321 and 333) were selected for the calculations.

Up-scaling to the stand level

In order to model shrub assimilation rates (Fa), we tested the significance of a series of climatic candidate variables (Tair, Tsoil, H%, PAR, SWC and VPD) measured on a 30-min basis in predicting Fa. PAR and VPD were always highly significant at $P < 0.001$. Fa estimates were obtained by a non-linear least square fit of the data to a rectangular hyperbolic light response curve:

$$\mathrm{Fa} = \frac{\alpha\beta Q}{\alpha Q + \beta} \quad (1)$$

where Fa is CO_2 assimilation rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), α is the initial slope of the light response curve, which is an approximation of the LUE, β is the maximum Fa rate (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), and Q is the photosynthetic photon flux density (μmol photon$\cdot m^{-2} \cdot s^{-1}$). To account for the influence of dry air on canopy conductance, we included an algorithm that takes into account the effect of VPD on Fa, where the β coefficient varies according to VPD (Lasslop et al. 2010):

$$\beta = \begin{cases} \beta = \beta_0, & \mathrm{VPD} < \mathrm{VPD}_0 \\ \beta_0 \exp(-k(\mathrm{VPD} - \mathrm{VPD}_0)), & \mathrm{VPD} \geq \mathrm{VPD}_0 \end{cases} \quad (2)$$

where VPD is vapour pressure deficit measured in the atmosphere (in hPa) and VPD_0 is set up as 10 hPa according to Lasslop et al. (2010). Models were fitted using a free and open-source software package for the development of nonlinear statistical models (AD Model Builder; Fournier et al. 2012). For up-scaling to the stand level, 30-min values of Fa estimates were obtained using the VPD measured in the under-canopy (VPDuc) and the photosynthetically active radiation under-canopy (PARuc) for the days of shrub gas exchange measurements and then integrated over a daylight basis. We used biomass distribution described above for extrapolation to the footprint area and compared the estimated shrub assimilation rates with the daylight GPP estimated from the partitioning of net ecosystem productivity from the eddy-covariance flux tower installed above the tree canopies. Only the days with high-quality data (quality flags and gap-filling) were used for this comparison.

Fluxes footprints were estimated for the period between DOY 172 and 329 using the ART footprint tool (Neftel et al. 2008), in which the input values are measurements made by the eddy-covariance system (wind direction and speed, SD of lateral wind speed, Obukhov length, friction velocity and measurement height). The diurnal fluxes during this period are predominantly from cork oak woodland with shrubs (average 44.2%). To perform comparisons between the eddy-covariance and

the up-scaled flux chamber measurements, we chose flux tower days that were closest to the measurement dates and that had over 60% of the footprint within the measurement area.

Statistical analysis and model performance

To examine differences among species we used one-way ANOVA. We used the paired *t*-test to examine variable changes in each species on a temporal scale. When statistically significant differences were found, differences between group means were identified by *post-hoc* Tukey HSD tests. When ANOVA assumptions where not met, namely normal distribution of the data and homogeneity of variances, non-parametric tests were carried out, performing a comparison on ranks, and Dunn's test was used for *post-hoc* pair-wise comparisons. The Pearson product moment correlation coefficient was used to display the strength of the association between pairs of variables. All statistical relationships were considered significant at $P < 0.05$. All statistical analyses were carried out using SigmaStat (SigmaStat for Windows v. 3.5; Dundas Software, Germany).

Statistical analysis of the models used in this study followed the approach proposed in Miehle et al. (2006) and Simioni et al. (2008). We examined the accuracy and the bias of predictions and observations on an average basis through calculation of the relative mean error of prediction ($\bar{e}\%$):

$$\bar{e}\% = 100\frac{\frac{\sum_{i=1}^{n}(O_i - P_i)}{n}}{\bar{O}} \quad (3)$$

where O_i and P_i are the observed and predicted values, $\bar{O}$ is the average of observations and n is the number of measurements. To account for the significant of prediction errors, we used the difference between the relative mean square root error of prediction (RMSE%) and the mean absolute relative error of prediction (MAE%):

$$\mathrm{MAE}\% = 100\frac{\sum_{i=1}^{n}|P_i - O_i|}{n\bar{O}} \quad (4)$$

$$\mathrm{RMSE}\% = \frac{100}{\bar{O}}\sqrt{\frac{\sum_{i=1}^{n}(P_i - O_i)^2}{n}} \quad (5)$$

We used a modification of the Nash–Sutcliffe index to obtain a quantitative measure of agreement between the predictions and the observations indicating the level of precision. This modification was suggested by Simioni et al. (2008) and provides a more apparent method to assess general model accuracy than the original formulation:

$$\mathrm{ME} = 1 - \frac{\sum_{i=1}^{n}|O_i - P_i|}{\sum_{i=1}^{n}|O_i - \bar{O}|} \quad (6)$$

Results

Climate

The meteorological conditions during 2011 (Fig. 1) were abnormal compared with the long-term averages. April and May recorded a total of 218 mm rain, double the average of 108 mm observed in the same period for 1971–2000. On the other hand, summer was relatively dry, with one large rain pulse of 22 mm (around DOY 240) at the beginning of September (Fig. 1a) and lasted longer than normal. Maximum VPD values in the first 2 wk of October 2011 (Fig. 1c) were three times higher (36 hPa) than values observed for the same period in 2010 (12 hPa). The drought period continued from summer throughout early autumn, and the first consecutive autumn rains were only recorded in November (Figs 1a, 2a for the days of chamber measurements).

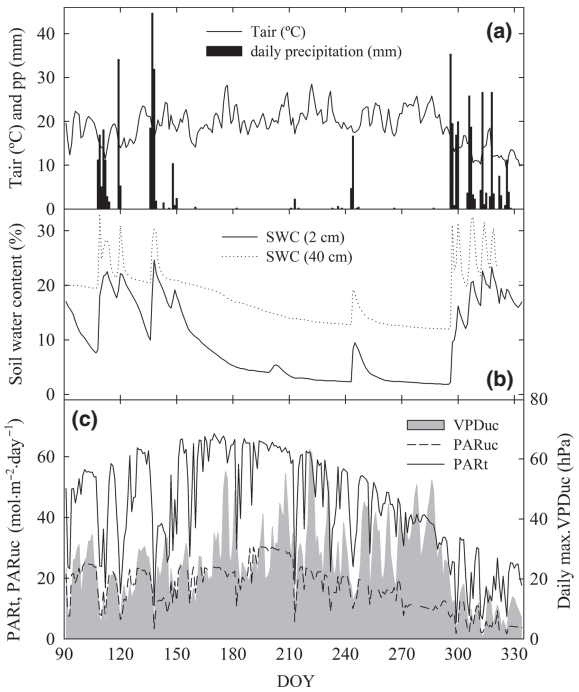

Fig. 1. Daily meteorological conditions during 2001. (**a**) Daily precipitation (pp, mm) and daily average air temperatures (Tair, °C). (**b**) Soil water content (SWC, %) at 2- and 40-cm depth. (**c**) Total photosynthetically active radiation (PARt, mol·m^{-2}·d^{-1}), under-canopy PAR (PARuc, mol·m^{-2}·d^{-1}) and maximum daily VPD measured in the under-canopy (VPDuc, hPa).

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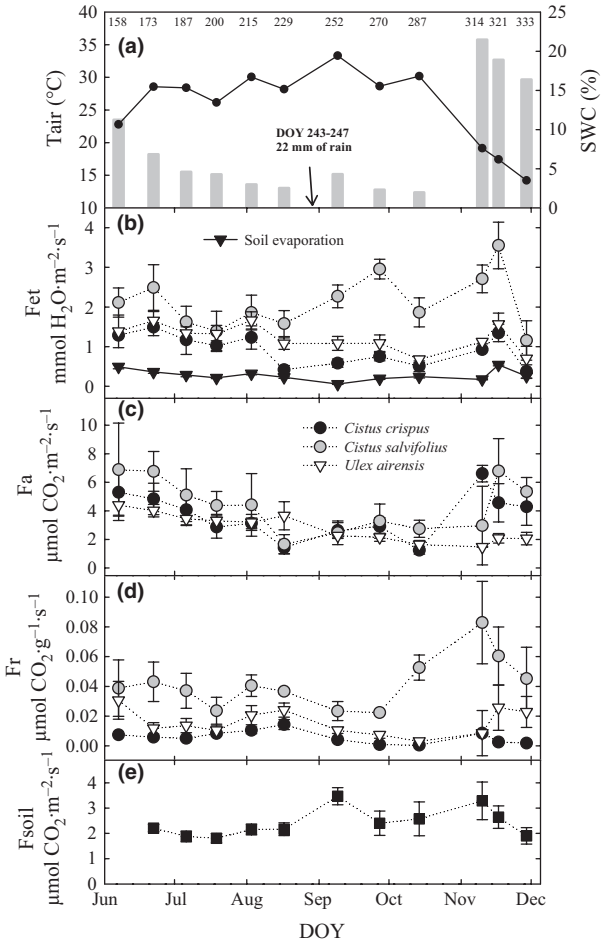

Fig. 2. General meteorological understorey conditions and gas exchange fluxes for the three shrub species measured from June until November 2011. (**a**) Soil water content at 2-cm depth (SWC, %, grey bars), air temperature (Tair, °C, continuous line). (**b**) Transpiration per shrub species on a leaf area basis (Fet, mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) and soil evaporation on an area basis (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$). (**c**) Net assimilation rates per shrub species (Fa, µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) on a leaf area basis. (**d**) Above-ground biomass respiration (Fr, µmol $CO_2 \cdot g^{-2} \cdot s^{-1}$) on a plant dry mass basis. (**e**) Soil respiration (Fsoil, µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). Julian days are shown in the top *x*-axis. Error bars represent SE, with $n = 2–4$.

Relative soil water content (SWC) followed closely precipitation events and decreased sharply after May (around DOY 150) reaching the lowest values at 2-cm depth (ca. 2.5%) in midsummer. At 40-cm depth, SWC showed a lower decrease and maintained values above 12% during summer and until the autumn rains (Figs 1b, 2a for days of chamber measurements).

The understorey received on average 36% of the PAR measured in the top canopy (Fig. 1c).

Shrub and soil gas fluxes

Water fluxes

Plant transpiration (Fet)

Shrub transpiration (Fet) decreased moderately (Fig. 2b) in early summer following soil water availability, and then varied little among species until the 22-mm rain event. This soil rehydration led to a prominent Fet increase in *C. salviifolius*, while the other two species only slightly increased Fet. In general, summer caused a significant decrease in Fet in all species, and *C. crispus* had a statistically significant lower Fet ($P < 0.001$) than the other shrubs from August to October.

Carbon fluxes

Photosynthesis (Fa)

The evergreen *U. airensis* had consistently lower assimilation rates during the study period compared with the semi-deciduous *Cistus* spp. Although *C. salviifolius* presented the highest Fa rates, it also showed higher variability. On a leaf area basis, differences among the three species were not statistically significant, except in autumn and between *U. airensis* and *Cistus* spp.

The onset of the drought season led to a significant leaf area reduction in semi-deciduous *Cistus* spp., with a concomitant decrease in assimilation rates (Fa). A drop of 60% in leaf assimilation rates was observed, on average, in *Cistus* spp. from DOY 215 to 229, while *U. airensis* maintained similar values. All species presented the lowest Fa rates in summer, although the 22-mm rain pulse from DOY 243–247 led to a slight photosynthesis increase in *Cistus* spp. while *U. airensis* continuously decreased. After DOY 270, the combination of very low soil moisture and higher VPD (>40 hPa; Figs 1c) led to the lowest assimilation rates in *Cistus* spp. and *U. airensis*.

The onset of the rainy season led to an increase in assimilation rates for *Cistus* spp., while *U. airensis* was practically unresponsive throughout the autumn ($P > 0.05$). *C. crispus* had an earlier response to water availability than *C. salviifolius*, which only on DOY 321 showed a marked Fa increase. *C. salviifolius* showed high variability in the observations, so the fluctuations before and after the rain pulse led to differences lacking statistical significance.

Plant respiration (Fr)

Shrub respiration was significantly higher ($P < 0.05$) in *C. salviifolius* than the other two species (Fig. 2d), which had similar respiration rates during the summer. Respiration rates were relatively constant until mid-August and then decreased until DOY 287, with the exception of *C. salviifolius*, which presented a pronounced increase (Fig. 2d). With the onset of rain *C. salviifolius* respiration increased greatly, while *C. crispus* was practically unresponsive. *U. airensis* respiration increased moderately.

Soil respiration (Fsoil)

Soil respiration remained constant (ca. 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) throughout the dry season (Fig. 2e) and clearly responded to the rain event in September (DOY 252). This soil rehydration was sufficient to stimulate soil microorganism activity and possibly some root activity, as Fsoil never dropped below 2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the rest of the dry season and until the first rains in November. This rain pulse effect was also noticed in the eddy-covariance measurements as an increase in ecosystem respiration on DOY 252 (Fig. 4).

Shrub morphology and physiology

The three shrub species showed contrasting morphological and physiological traits (Table 2). Among the species, *U. airensis* has the highest leaf area as well as the highest investment in root system development, with a root/shoot ratio of 0.68 ± 0.04. This species has a much deeper root system than the other two, exploring the soil beyond 40-cm depth. Conversely, *C. salviifolius* has a lower leaf area and the lowest investment in root development, with a root/shoot ratio of 0.29 ± 0.06; noteworthy, this species has a very shallow root system restricted to the first 15-cm soil layer but with a very dense distribution and the highest root surface area per volume of soil. Also, *C. salviifolius* has a higher SLA than *C. crispus*, denoting a better adaptation to high light conditions, and grows taller than *C. crispus*. In general, *C. crispus* presented intermediate values in leaf area and root development.

Under summer drought stress, *U. airensis* had a significantly ($P < 0.001$) higher Ψ_{pd} than both *Cistus* spp. (Table 2), confirming its higher capacity for soil water absorption due to its deeper root system.

Table 2. Morphological and physiological traits per species: total plant biomass, specific leaf area, leaf area, root/shoot, root surface area/volume of soil, root distribution, summer predawn leaf water potential (Ψ_{pd}), total chlorophyll and chlorophyll *a/b*. ES is early summer, SE is summer end and MA is mid-autumn.

Characteristics		*Ulex airensis*	*Cistus crispus*	*Cistus salviifolius*
Total plant biomass (g)		60 ± 24.8	29.1 ± 5 1	13.4 ± 2.3
Specific leaf area ($m^2 \cdot kg^{-1}$)		7.9 ± 0.47	8.9 ± 0 28	11.5 ± 1.71
Leaf area (m^2)		0.12 ± 0.05	0.06 ± 0.01	0.03 ± 0.01
Root/shoot		0.68 ± 0.04[a]	0.45 ± 0 10[ab]	0.29 ± 0.06[b]
Root surf./vol. soil ($m^2 \cdot m^{-3}$)		1.02 ± 0.3	0.79 ± 0.2	1.16 ± 0.3*
Maximum root depth		>60 cm	40–50 cm	10–20 cm
General plant morphology (example from harvested plants)				
$SE\Psi_{pd}$	(MPa)	−1.4 ± 0.2	−2.5 ± 0.1	−2.9 ± 0.3
Total chlorophyll ($mg \cdot g^{-1}$)	ES	4.11 ± 0.8[a]	8.1 ± 1.18[b]	6.42 ± 1.11[b]
	SE	4.83 ± 0.53[a]	9.52 ± 0.88[b]	11.36 ± 0.48[b]
	MA	2.31 ± 0.15[a]	5.36 ± 0.55[b]	4.6 ± 0 25[b]
Chlorophyll *a/b*	ES	1.63 ± 0 17	1.02 ± 0.18[b]	1.51 ± 0.21
	SE	1.73 ± 0.11[a]	1.04 ± 0.13[b]	0.82 ± 0.07[c]
	MA	2.21 ± 0.02[a]	1.44 ± 0.12[b]	1.71 ± 0.23[b]

*Roots found only in the 15-cm top soil.

Different letters represent statistical significance at $P < 0.05$, no letters means no differences (average ± SE).

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Regarding chlorophyll content, total chlorophyll increased for all species from early summer to the end of summer, dropping sharply in the moist season. Total chlorophyll was two times higher in the semi-deciduous *Cistus* spp. compared to *U. airensis*. Regarding chlorophyll *a/b*, there was a clear decrease for *C. salviifolius* from early summer to the end of summer, resulting from chlorophyll *a* degradation, but *U. airensis* and *C. crispus* were able to maintain the ratio. A recovery occurred in mid-autumn for all species, but was more pronounced for the *Cistus* spp.

Water, light and carbon use efficiencies

All three species were able to maintain similar WUE from early summer to the end of summer (Fig. 3a). Soil water rehydration in autumn, together with a significant drop in VPD, led to an increase in WUE in all species except *U. airensis*, in which it only moderately increased. This WUE increase for *Cistus* spp. was the result of canopy photosynthesis recovery with a high control of water loss. Nevertheless, *C. salviifolius* presented a modest increase in WUE in mid-autumn (Fig. 3a), resulting from a proportionally higher transpiration compared with recovery in assimilation rates (Fig. 2b,c).

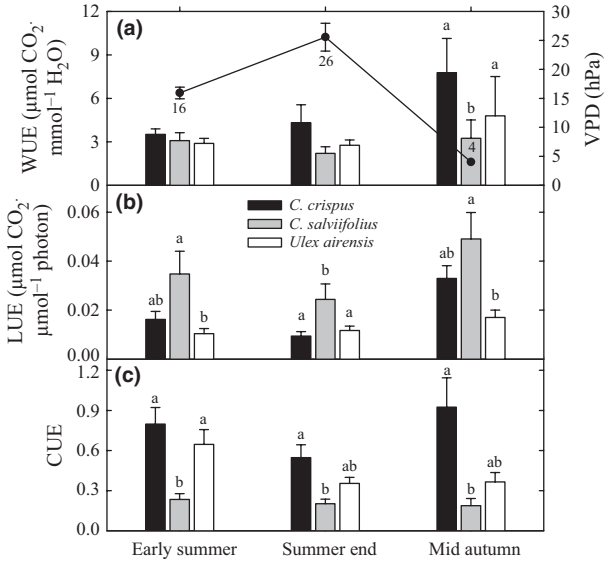

Fig. 3. Temporal variation in water, light and carbon use efficiencies for *Cistus crispus* (black bars), *C. salviifolius* (grey bars) and *Ulex airensis* (white bars). (**a**) Water use efficiency (WUE, μmol CO_2 $mmol^{-1}$ H_2O) and average max. daily VPD (hPa). (**b**) Light use efficiency (LUE, μmol CO_2·μmol^{-1} photon), (**c**) carbon use efficiency (CUE, dimensionless). Letters represent significant differences ($P < 0.05$) between species for each period.

Throughout the summer, LUE was higher in *C. salviifolius* when compared to the other two species (Fig. 3b). No differences were observed at a seasonal level, although *C. crispus* increased LUE after the first rains mainly due to a recovery in assimilation rate. On the other hand, *C. crispus* always showed a significantly higher CUE than *C. salviifolius* (Fig. 3c), which was mainly due to increased differences in respiration rates during the summer and autumn (Fig. 2d). The drought at the end of summer was accompanied by a decrease in CUE for *C. crispus* and *U. airensis*, followed by a recovery in mid-autumn only for *C. crispus*.

Up-scaling to the stand level

Modelling results

The inclusion of VPD limitation improved the model ability to predict instantaneous Fa for *C. crispus* and *U. airensis* but not for *C. salviifolius*.

The results show that model bias (given by *e*%) was considerably higher for *C. salviifolius* with the inclusion of VPD, meaning that the predictions were under-estimated by an average of 56%. The difference between RMSE% and MAE%, which reflects the existence of significant prediction errors, also corroborates this result. For *C. crispus* and *U. airensis*, the inclusion of VPD in the models decreased the error bias and increased model efficiency.

Eddy-covariance measurements and shrub up-scaling

Eddy-covariance data showed that the ecosystem was a strong carbon sink during the early summer (Fig. 4). This carbon sequestration decreased gradually with the onset of the dry season, although a positive carbon balance was maintained until the first rains in mid-autumn. The

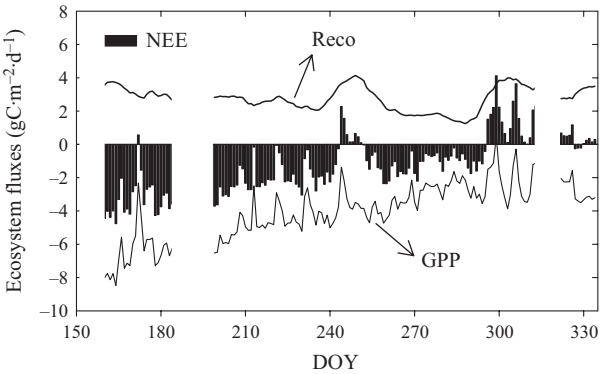

Fig. 4. Net ecosystem exchange (NEE), gross primary productivity (GPP) and ecosystem respiration (Reco) measured using eddy-covariance. Discontinuities in the data are due to rejected data in filtering and/or gap-filling processing.

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22-mm rain event in early September caused an ecosystem CO_2 efflux to the atmosphere, mostly due to a soil respiration pulse (Fig. 2e) as a consequence of soil rehydration.

Based on chamber and up-scaling of gas exchange measurements, the shrub contribution to ecosystem CO_2 uptake (GPP) was, on average, 17%. Due to a higher proportion in the understorey (see total C stock estimates in Table 1), *C. salviifolius* made a particular high contribution of 14%, while *C. crispus* and *U. airensis* only amounted to 2% each. This CO_2 uptake contribution follows an expected seasonal trend in accordance with soil water availability and assimilation rate limitation. Therefore, in the early summer, the average contribution was 7%, increasing to 10% with drought stress at the end of summer. After the first rains in mid-autumn, there was an assimilation rate recovery (Fig. 4), and the contribution to GPP peaked at 35%.

Discussion

The understorey of this cork oak woodland comprises two functional groups (semi-deciduous and evergreen), which have developed different strategies of using light, water and carbon to overcome summer drought and compete successfully under the tree canopies.

Leaf traits, root depth and its relation with summer resource exploitation

Cistus salviifolius

Cistus salviifolius exhibited higher rates of net photosynthesis throughout the study period, which was related to canopy architecture. Leaves are arranged in many layers, with longer and perpendicular branches at the base decreasing in length and angle towards the top, thus optimizing light capture and reducing self-shading (see Table 2; Givnish 1988; Pearcy et al. 2005). Werner et al. (1999) also found that *Cistus* spp. are able to structurally adjust leaf orientation in accordance with water and light availability, which confers some advantage in heterogeneous shade conditions such as in this understorey environment. We also observed that *C. salviifolius* presented summer dimorphic leaves: one group of fully expanded leaves formed in early spring that are mostly lost in summer and another group of small leaves (around 30%) formed in late spring that remain small and attached to the plant during summer. This feature was also observed in other semi-deciduous Mediterranean shrubs (Kyparissis & Manetas 1993; Aronne & De Micco 2001). The smaller leaves contained five times more chlorophyll per unit dry mass than adjacent old leaves, suggesting plant resources mobilization to the maintenance of the photosynthetic apparatus. Maintaining these leaves is advantageous to *C. salviifolius* when the first autumn rains start, as they quickly expand and start to photosynthesize prior to other co-occurring species (Harley et al. 1987). So it is likely that these small leaves may be contributing to the higher assimilation rates observed.

The way in which *C. salviifolius* optimizes light harvesting and uses it to maintain higher photosynthetic rates is in accordance with the higher LUE observed (Fig. 3b). Additionally, light response curves and gas flux data from light chambers are consistent with the finding that *C. salviifolius* always achieves higher photosynthetic rates irrespective of light available and soil drought status. In relation to CUE, *C. salviifolius* presented the lowest values (Fig. 3c) even if it had higher photosynthetic rates. This low efficiency in using the assimilated carbon is mainly due to the very high respiration cost throughout the season and is associated with the maintenance of aboveground components (Fig. 2d). The high success of *C. salviifolius* in the understorey of this cork oak woodland, representing almost 70% of the understorey shrubby carbon stock, may be related, to some extent, to these two features: a higher capacity to exploit the reduced light available under the tree canopy and the ability to exploit water in an opportunistic way due to its shallow roots.

Both semi-deciduous *Cistus* spp. experienced a significant reduction in leaf area, reaching 62% in *C. salviifolius* and 45% in *C. crispus*. Leaf area reduction is a well-known drought stress adaptation in semi-deciduous species that greatly reduces leaf transpiration (Harley et al. 1987; Wedler et al. 1996). On a leaf area basis, this water loss decrease was modest in both *Cistus* spp., probably because spring was wet and moderate levels of soil water were maintained at the beginning of summer. Nonetheless, the two species showed clear and distinct responses after the

Table 3. Parameter values for the hyperbolic light models tested for each species with and without the VPD limitation. Parameters are described in Eqs. (1) and (2). Statistical outputs and model performance tested using the relative mean error of prediction ($\bar{e}$%), the difference between the relative mean square-root error of prediction (RMSE%) and the mean absolute relative error of prediction (MAE%) and model efficiency (ME).

With VPD	Parameter estimates			Model performance		
	β_0	α	k	$\bar{e}$%	RMSE% − MAE%	ME
C. crispus	8.463	0.030	0.051	1.4	12.3	0.26
C. salviifolius	6.251	0.153	0.042	56.2	23.1	−0.07
U. airensis	4.184	0.029	0.043	7.1	18.7	0.12
Without VPD	β	α				
C. crispus	4.550	0.039		7.6	14.9	0.16
C. salviifolius	4.949	0.123		10.5	18.1	0.04
U. airensis	3.844	0.030		7.17	18.8	0.09

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22 mm rain pulse in late August (Fig. 2b), as discussed below. Excluding this rain pulse effect from the analysis, *C. salviifolius* was able to maintain the same water use efficiency from early summer to the end of summer, even with an increase of 60% in VPD. Autumn rains led to only a modest recovery of photosynthesis in *C. salviifolius*, which was unexpected for a shallow-rooted shrub which, according to Harley et al. (1987), responds quickly to rainfall.

Ulex airensis

Ulex airensis presented lower photosynthesis per unit leaf area but more homogeneous rates during the studied period (Fig. 2c). Concomitantly, respiration rates remained low, leading to an intermediate CUE when compared to deciduous species, possibly related to the higher cost of leaf construction and maintenance (see Table 3 for SLA values in *Ulex* and *Cistus*; Walters & Reich 1999; Baldocchi et al. 2010). Recovery of photosynthesis and growth only occurs later in spring, when soil moisture and air temperature are not limiting.

During the experiment, *U. airensis* only showed modest signs of senescence at the peak of stress in the lower canopy (average 14% of total above-ground biomass). Lower branch shedding can have several purposes. One is to maximize light penetration in the inner canopy, as *Ulex* tends to produce very dense canopies resulting from the high accumulation of woody tissue (see Table 2 for above-ground biomass; Valladares et al. 2003). This may partly explain the lower LUE found. According to Tyree et al. (1993), this is a mechanism to prevent the whole plant from hydraulic failure by sacrificing some less productive and shaded shoots and thus protecting the stem from cavitation. Finally, lower and old branch shedding can also be a carbon economy strategy in order to optimize the ratio between plant carbon gains and losses.

Increased leaf longevity in an evergreen like *U. airensis* requires a conservative strategy in terms of water and light use in order to avoid irreversible leaf damage (Tenhunen et al. 1990). Leaf transpiration rate was significantly lower in *U. airensis* than in *C. salviifolius* but was similar to *C. crispus*, meaning that *U. airensis* survives summer in a better hydrated condition. This is supported by drought stress peak predawn leaf water potentials, which were lower (more negative) in *C. salviifolius* than in *U. airensis* (Table 2). This latter species seems to have isohydric behaviour, maintaining relatively constant leaf water potential but reducing stomatal conductance as the soil dries out. This is consubstantiated in *U. airensis* by the root/shoot ratio and access of roots to soil deep water, as the roots easily extend beyond 60 cm soil depth (see Table 2). Nonetheless, the apparent control of transpiration rates in *U. airensis* does not yield higher WUE, meaning that all three species are equally efficient in using water from early summer to the end of summer.

Cistus crispus

This shrub showed a strategy of environmental resource utilization standing between *U. airensis* and *C. salviifolius* and was apparently more efficient. With lower carbon assimilation rates but also the lowest respiration rates (Fig. 2c,d), *C. crispus* presented a significantly higher CUE throughout the experimental period. It also showed high control on water loss leading to a higher (but not statistically significant) WUE. Also, tap roots and shallow feeder roots (Table 2) allow *C. crispus* to access both deep water supplies and surface precipitation, providing a higher ability to explore available water and therefore more control of leaf senescence and abscission during summer. The corrugated pubescent leaves must be contributing also for leaf transpiration inhibition. This hability may also explain the significant increase in WUE and CUE after the first autumn rains. Canopy architecture in *C. crispus* seems to be consistent with the statistically lower LUE found for this species: the plants are stocky, grow in the shade of other plant canopies and the leaves are packed into one layer with a high level of self-shading (Table 2).

Response to a summer rain pulse: when opportunistic strategies do not compensate

A 22-mm rain event occurred at the end of August, followed by a long period of hot and dry conditions (September and October). Species responded differently to this sudden soil water availability. This rain event moistened the soil up to a depth of 40 cm but had returned to pre-rain values after 10 d (Fig. 1b) so it mostly benefited the species with shallow roots. *C. salviifolius* was particularly responsive, showing an increase in assimilation rates related to the expansion of the small summer leaves. Average canopy total chlorophyll per unit dry mass in leaves just before and after the rain event increased 17% in *C. salviifolius* from 9.7 to 11.4 $mg \cdot g^{-1}$, indicating canopy photosynthetic pigment recovery. Conversely, chlorophyll content only slightly increased in *C. crispus* (10%) and remained stable in *U. airensis*, reinforcing the relative insensitivity of this latter species to summer rain pulses.

A rapid leaf sprouting response to soil moisture is a common feature of semi-deciduous species. This is advantageous once the autumn rains start because nutrient and water uptake occur prior to that of neighbouring species (Harley et al. 1987; Kyparissis & Manetas 1993; Grammatikopoulos 1999). This allows plants to reach earlier maximum leaf area, thus optimizing carbon assimila-

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tion. However, the ecophysiological implications are not the same when a sporadic summer rain event is followed by long and dry conditions. Thus, *C. salviifolius* did not shown any sign of stomatal control over water loss, as the soil dried out after the rain pulse, contradicting the findings of Harley et al. (1987), who reported considerable stomatal control of transpirational water loss in *C. salviifolius*. Although some photosynthesis still occurred (Fig. 2c), transpiration rates increased abruptly (Fig. 2b) and then later respiration also increased (Fig. 2d) leading to a plant carbon balance disequilibrium, in some cases irreversible, as field observations (not quantified) revealed plant death. Such a breakdown may be related to the maintenance respiration cost of the small, expanded leaves. In contrast, *U. airensis* and *C. crispus* did not respond to the rain event, confirming there 'play safe' strategy towards a carbon cost-effective equilibrium (Werner et al. 2001).

Summer rain pulses are known to stimulate soil microorganism activity due to sudden soil rewetting after a long dry period, where abundant partly decomposed organic matter from the previous spring is available for decomposition. This causes a rapid and pronounced CO_2 efflux to the atmosphere (Fig. 2e). However, there is also plant respiration feedbacks to this rain event. In this study, the overhasty response of *C. salviifolius* to immediate soil moistening ended in a huge above-ground plant carbon efflux that may have also contributed to ecosystem carbon lost in summer. We may speculate that, under a future climate change scenario of longer drought periods punctuated by extreme rainfall events, survival of *C. salviifolius* may be threatened. However, only a detailed analysis of the various aspects of plant strategy, namely growth patterns and reproduction, would allow elucidation of a more comprehensive scenario for the development of this species.

Shrub contribution to ecosystem CO_2 uptake

Except for *C. salviifolius*, the inclusion of VPD data in models helped to increase their accuracy in estimating Fa (Table 3), with a decrease in carbon assimilation rates when temperature and VPD are higher (Pettigrew et al. 1990; Lasslop et al. 2010). Nevertheless, the approach used failed to accurately describe the high variability in measured carbon assimilation rates, especially for *C. salviifolius*. This variability may be partly explained by the highly dynamic understorey light environment, punctuated by brief and unpredictable periods of direct solar radiation (sun flecks). Shade species are able to efficiently use these sun patches but can also become susceptible to photoinhibition during periods of drought when CO_2 diffusion inside the leaf is limited by low stomatal conductance (Chaves et al. 2004). Diffuse light is known to provide higher assimilation rates, as it is used more efficiently (Rosati & Dejong 2003), which means that higher Fa does not necessarily coincide with high PAR and lower VPD. An analysis of the sources of error revealed that the models failed to estimate the highest Fa values by default, meaning that our daily estimates are rather conservative. We consider that only a model specifically addressing species-specific photosynthesis optimization under shade and drought would provide a statistically stronger up-scaling exercise.

The average contribution of the understorey GPP to total ecosystem CO_2 uptake was estimated at 17%, very similar to the rates of 20% found by Wedler et al. (1996) in the understorey of a Scots pine plantation, and 25% found by Sakai et al. (2006) in a cool-temperate deciduous broadleaf forest in central Japan. Furthermore, the values found in this study are within the range of 0–39% reported by Misson et al. (2007) for several forest ecosystems. The 7% contribution of the understorey during early summer results from the proportionally higher contribution of the tree component to ecosystem GPP compared with that of the shrubs. Early summer is the time when cork oaks are physiologically more active: their canopies have new shoots and leaves and their deep roots have access to ground water tables (Chaves et al. 2004; David et al. 2007). We speculate that the slight increase in shrub contribution at the end of summer may result from the more favourable understorey environment for the shrubs at the peak of summer, with lower VPD and lower risk of photoinhibition. The higher shrub contribution occurred in mid-autumn (35%), which may be explained by the rapid shrub response to soil rehydration upon the onset of autumn rains (Fig. 2c), particularly from the two *Cistus* spp.

The 17% contribution of the shrubby understorey to ecosystem carbon sequestration shows that allowing shrub recruitment may increase forest mitigation importance in a climate change context. However, due to the extremely aggressive and competitive feature of Mediterranean shrubs, management of the shrubs growing beneath the trees should be a compromise between benefits and costs, especially in relation to competition with trees for water and nutrients and also the increased fire risk. This is still a research gap in Mediterranean forest management.

Besides the ecological services provided by a shrubby mosaic, namely protection from soil erosion, a biodiversity refuge and an increase in soil fertility (Bugalho et al. 2011), shrub recruitment management can help in ecosystem restoration. For example, the generalized lack of cork oak regeneration is considered one of the main problems of future sustainability of *montados* (Plieninger et al. 2010). Recent studies reveal that in stress-prone ecosystems such as those in the Mediterranean region, the heterogeneity generated by shrubs facilitates the establishment of tree species (Gomez-Aparicio et al. 2005). Tree seedling establishment and survival benefit significantly from modifica-

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tion of the abiotic environment promoted by nurse shrubs, where shading appears the crucial facilitating factor (Smit et al. 2008). Although this shrub–tree seedling beneficial interaction seems to be species-specific (Rolo & Moreno 2011), there is a broad general consensus that augmenting populations of nurse shrubs and trees should be considered a promising strategy for restoring woody late-successional communities (Valladares et al. 2003; Prider & Facelli 2004; Gomez-Aparicio et al. 2005; Smit et al. 2008; Plieninger et al. 2010; Rolo & Moreno 2011).

This study allows us to conclude that under a global change perspective, the future of cork oak forests requires a scientifically sound conservation strategy and locally tailored sustainable management that must accommodate various aspects of understorey plant biology and ecosystem functions.

Conclusions

The understorey shrubby vegetation of this *montado* ecosystem contributed 17% to ecosystem net CO_2 uptake, reinforcing our hypothesis of the importance of shrub recruitment in forest mitigation in a climate change context. The mixture of sclerophyllous plants that naturally colonize the understorey, although with contrasting leaf habits and rooting depths, was able to balance carbon gainsand water loss during the most critical time in the Mediterranean climate: the summer drought. However, the unexpected summer rain pulse in the middle of the study allowed us to distinguish between species' ability to use water that is immediately available and its impact on whole plant carbon and water balance. We conclude that shallow-rooted, semi-deciduous species exploit environmental resources in an opportunistic way and are more susceptible to longer dry periods following summer rain pulses. This study reinforces that Mediterranean rain pulses are particularly detrimental to ecosystem carbon sequestration, not only due to soil respiration efflux but also due to plant respiration losses. Future studies should focus on understorey plant ecophysiological processes concerning environmental resource exploitation by different functional groups. Due to the difficulty in reproducing water and carbon fluxes, modelling exercises should specifically incorporate realistic photosynthetic responses to the patchy forest undertorey light, and also plant water and carbon efficiencies at a seasonal time-scale.

From a sustainable management standpoint, the study of shrub–tree interactions is essential as during drought, Mediterranean shrubs are extremely aggressive, competing directly with trees for water and nutrients. Shrub encroachment can greatly increase fire risk and might jeopardize the potential for long-term carbon sequestration.

Acknowledgements

This research was carried out with financial support from the following grants: SFRH/BD/39058/2007 for Correia and SFRH/PBD/46839/2008 for Costa e Silva. The authors thank Herdade da Machoqueira do Grou and Grupo Amorim for support with field facilities. We also thank Arndt Piayda from Leichtweiß Institute for field technical support. The contribution of four anonymous referees to improvement of the manuscript is greatly acknowledged.

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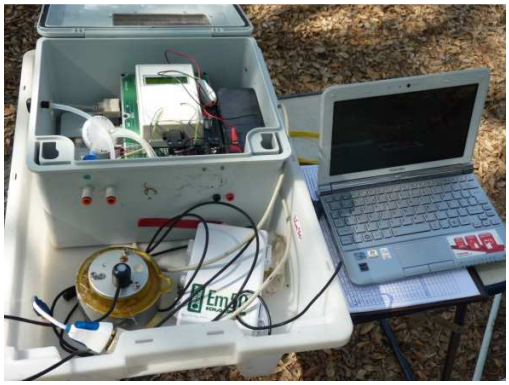
Doi: 10.1111/jvs.12102

Aspecto do sub-coberto arbustivo do montado de sobro na Herdade da Mochoqueira do Grou (Concelho de Coruche) com uma densidade de 170 árvores por ha

Amostragem destrutiva do sistema radicular em *Ulex airensis* (esquerda) e da biomassa acima do solo em *Cistus crispus* (direita)



Câmara de luz (esquerda), câmara escura (centro) e analisador de CO_2 e H_2O Li840a, da marca *Li-Cor* (direita)

Resumo

A respiração do solo em regiões de clima mediterrânico está fortemente relacionada com a sazonalidade da precipitação que se espera que venha a ser alterada num contexto de alteração climática.

Neste estudo utilizámos uma extensa colecção de medições de respiração do solo de florestas a pastagens realizadas ao longo de 10 anos na região centro e sul de Portugal. A análise dos dados evidenciou que a sazonalidade da respiração do solo é afectada pela humidade e temperatura do solo. A estatística Bayesian foi utilizada para testar a eficiência de modelos climáticos na estimação da Rs numa escala diária e mensal.

A sazonalidade da respiração do solo foi semelhante entre ecossistemas com um máximo na Primavera e Outono e um mínimo na estação seca (Julho a Setembro). Não foram observadas diferenças para a respiração do solo entre sites com diferentes densidades de árvores ou teores de carbono do solo quer numa escala sazonal ou anual. A humidade do solo, e não a temperatura, foi a variável que melhor explicou os efluxos de CO_2 do solo durante grande parte do ano. A temperatura explicou variações na respiração do solo apenas acima de determinados limites de humidade: 10% para os ecossistemas florestais e 15% para as pastagens, conduzindo a um Q_{10} de 2.01, 1.61 e 1.31 para povoamentos de densidade elevada, de densidades baixas e pastagens, respectivamente. A estaística Bayesiana demonstrou que os modelos com inclusão da humidade do solo como variável dependente apresentaram um melhor desempenho do que os modelos baseados unicamente na temperatura. É possível obter estimativas mensais da respiração do solo nas pastagens utilizando modelos simples baseados na humidade do solo mas nenhum deles se adequa para estimativas da respiração do solo em povoamentos florestais sugerindo, neste caso, a necessidade de uma abordagem mais processual.

Este estudo vem reforçar a importância da humidade do solo no controlo dos efluxos de CO_2 dos solos da região mediterrânica, variável esta que não deve ser negligenciada em exercícios de modelação do balanço de carbono ao nível do ecossistema.

Abreviaturas

μ	mean
σ	standard deviation
μ^*	distribution mean of the absolute values of the elementar effects
BMC	Bayesian model comparison
CF	Closed forest
D	Observed data
Hs	Soil water contente (%)
L%	Percentage model probability suitability
LAI	Leaf area index (m^2 m^{-2})
MG	Mediterranean grassland
Mi	Output from the model
MSE	Mean squared error
NRMSE	Normalized root mean squared error
OF	Open forest
$P(D\|\theta)$	Likelihood
$P(M_k\|D)$	Integrated likelihood
$P(\theta)$	prior
$P(\theta\|D)$	Posterior
pp	total annual precipitation (mm)
Q_{10}	temperature sensitivity of soil respiration
Rs	Soil respiration (μmol CO_2 m^{-2} s^{-1})
Ts	Soil temperature (ºC)

Agriculture, Ecosystems and Environment 161 (2012) 88–100

Contents lists available at SciVerse ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Soil water availability strongly modulates soil CO_2 efflux in different Mediterranean ecosystems: Model calibration using the Bayesian approach

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ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 23 July 2012
Accepted 24 July 2012

Keywords:
Soil CO_2 efflux
Soil respiration
Mediterranean
Bayesian calibration
Empirical model
Soil moisture
Soil temperature

ABSTRACT

Soil respiration in drought prone regions is highly dependent on the precipitation regime and soil moisture conditions, which are expected to change in a global warming context. In the present study we used an extensive collection of field chamber measurements of soil respiration (R_s) from forest and grassland sites of centre and south of Portugal distributed over a 10 year period. This data were summarized and analysed with the objective to describe seasonal variability of R_s as affected by soil moisture (H_s) and soil temperature (T_s). A Bayesian framework was used to test the effectiveness of soil bioclimatic models in estimating R_s on a daily and monthly time step. R_s seasonality was similar between sites, reaching a maximum in spring and autumn and a minimum in the dry season (July–September). No differences were observed for R_s between sites with different standing biomass or soil carbon stocks either on an annual or seasonal timescale. H_s, and not T_s, was the driving factor of R_s during most of the year. T_s drove R_s response only above certain H_s limits: 10% for forest sites and 15% for grassland sites leading to a Q_{10} of 2.01, 1.61 and 1.31 for closed forests, open forests and grasslands, respectively. The Bayesian analysis showed that models using H_s as an independent variable performed better than models driven by T_s alone. Monthly estimates of R_s in grasslands can be predicted by simple climatic models based on H_s but none of them was suitable for forest ecosystems, stressing the need for a process-based approach. This study adds to the evidence that H_s controls R_s fluxes for Mediterranean ecosystems and should always be taken into account for extrapolation purposes.

1. Introduction

Soil respiration, R_s, is one of the most important fluxes in the terrestrial carbon (C) cycle. In 2008, global R_s was estimated at 98 ± 12 Pg C (Bond-Lamberty and Thomson, 2010), with an increase of 0.1 Pg C year^{-1} between 1989 and 2008, attributed to the increase in global air temperature recorded in the last decades.

Mediterranean regions in particular are presently facing an increase in the frequency and severity of droughts (Ramos et al., 2011), with potentially negative effects on the productivity of agricultural crops, pastures and forests. In such semi-arid ecosystems, plant productivity is largely controlled by climatic drivers, with water availability being among the most important. Such drought-induced decreases in primary productivity can also affect the total CO_2 efflux from soils impacting litter fall, decomposition and belowground allocation of photosynthates to roots and root turnover (Reichstein et al., 2002).

Droughts in Mediterranean systems occur during periods of high temperature and low soil moisture content. These environmental conditions also tend to reduce the metabolic activity of living tree roots, mycorrhizae and other rhizosphere microorganisms and soil fauna leading to reductions in CO_2 losses from soils (Hanson et al., 2000). Other physical and chemical processes, like soil CO_2 degassing and transport through the soil profile and the chemical oxidation of soil minerals, are considered minor contributors to the net CO_2 efflux (Buchmann, 2000).

Soil temperature is one of the most influential and widely studied factor affecting R_s (Lloyd and Taylor, 1994; Kirschbaum, 1995; Yuste et al., 2004; Zhou et al., 2009). In general, R_s increases with T_s, and its relationship is usually described with exponential equations (see Webster et al., 2009 for examples). The temperature

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http://dx.doi.org/10.1016/j.agee.2012.07.025

dependence of R_s, that is the factor by which a variable changes for a temperature increase of 10 °C (Q_{10}), ranges from 1.3 to 5.6 (Lloyd and Taylor, 1994), although Mahecha et al. (2010) suggest that this factor is confined to values around 1.4 ± 0.1. This Q_{10} factor, however, is only valid for a limited range of temperatures and under drought conditions temperature plays a secondary role in explaining R_s (Kirschbaum, 1995; Janssens and Pilegaard, 2003; Lenton and Huntingford, 2003; Yuste et al., 2004; Almagro et al., 2009).

In ecosystems that are prone to drought, T_s and H_s often interact to control R_s, with R_s responding to the most limiting factor. In summer, R_s significantly decreases with water stress because of a decline in plant metabolic activity and an inhibition of soil microbiological carbon decomposition (Jarvis et al., 2007). When water is not limiting, R_s generally increases with temperature (Raich and Schlesinger, 1992; Inglima et al., 2009). This partially explains why attempting to model R_s using only temperature driven variables proved ineffective in drought-prone regions (Joffre et al., 2003; Xu and Baldocchi, 2004; Almagro et al., 2009; Migliavacca et al., 2011).

While some authors argue that purely climatic driven models are sufficient for accurately predicting R_s (Raich and Schlesinger, 1992; Raich et al., 2002), others emphasise the need to separate climatic and biological effects on R_s (Reichstein et al., 2002, 2003). Although there have been many attempts to incorporate biotic variables in models, e.g. using substrate quantity and quality (Webster et al., 2009), leaf area index (Reichstein et al., 2003) and productivity (Irvine et al., 2005), climatic driven models are still widely used with success (Rey et al., 2002; Conant et al., 2004; Tang et al., 2005; Almagro et al., 2009; Webster et al., 2009; Zhou et al., 2009). This is in part because climatic driven models are simpler to use and less input demanding. Nonetheless, fitting empirical models with proxy parameters of biological activity can yield very good results when enough information is available (Migliavacca et al., 2011).

The majority of *R*s data is collected using portable field chambers. One fundamental problem of ecological model application is scaling-up results from these small chambers to the stand or ecosystem level. Sporadic measurements of *R*s have obvious limitations as they may not accurately capture the temporal and spatial variability or the dynamic nature of the C release from roots and microbes. In this sense models are important as they allow the estimation of these processes at larger scales. Models need to be calibrated and validated in order to apply them robustly, and one powerful tool to evaluate model applicability and performance is using Bayesian statistics. This approach allows the calibration and the quantification of uncertainty for models that contain multiple parameters (Kass and Raftery, 1995; van Oijen et al., 2011; Sivia and Skilling, 2006).

The objectives of this study were: (1) to assess the seasonal variability of R_s among vegetation types; (2) to quantify the effects of T_s and H_s on R_s in different Mediterranean ecosystems; (3) to evaluate, under a Bayesian framework proposed by van Oijen et al. (2011) and improved by Minunno et al. (submitted for publication), the performances of climate driven models in predicting R_s. We used a long-term database spanning 10 years of R_s measurements, covering grasslands and open and closed forests, using the same field-portable device, a close dynamic system EGM-1 from PP Systems (PP System, Amesbury, USA). This allowed intersite comparisons reducing systematic errors associated with the equipment (which avoided the need for cross calibration) and measurement technique (which was the same for all sites). This work is innovative because it applies a Bayesian approach for model comparison and for global sensitivity analysis to R_s models in Mediterranean regions, which had only been applied by Tuomi et al. (2008) and Hashimoto et al. (2011) in boreal and temperate regions.

2. Materials and methods

2.1. Site description

The experimental sites are located in the centre and south of Portugal. All sites are characterized by a Mediterranean climate with more than 80% of total annual precipitation occurring between October and April. Average annual precipitation (long-term averages from 1971 to 2000) is 608 mm with a mean annual temperature of 15.9 °C. Soils have sandy silt or sandy clay textures (see Table 1 for other site characteristics).

The eight sites have different characteristics that were grouped accordingly to the type of vegetation cover: closed forests (CF), open forests (OF) and Mediterranean grasslands (MG). The CF vegetation type includes a eucalyptus (*Eucalyptus globulus*) stand (CF1) with 1110 trees ha^{-1} and a young and dense evergreen cork oak (*Quercus suber*) woodland site (CF2) with 424 trees ha^{-1}. A pine (*Pinus pinea*) forest (OF1) with 120 trees ha^{-1} and two evergreen oak (*Q. suber* and *Quercus ilex*) sites with 55 (OF3) and 180 trees ha^{-1} (OF2) were included in the open forest cover type (OF). The Mediterranean grasslands (MG) included a natural pasture dominated by winter-spring C3 annuals (MG3), an improved pasture, seeded with a legume-rich seed mixture (MG2) and a C3 grassland with scattered shrub cover of *Cistus* sp. (MG1).

2.2. Soil respiration measurements

Soil respiration (R_s) was measured at each site for a minimum period of 6 months covering the growing season, at weekly or fortnightly intervals. In each site, R_s was measured with the same device, a soil respiration chamber (EGM-1, PP Systems, Hitchin, UK), that uses an infrared gas analyser to measure the rate of CO_2 accumulation inside a closed chamber system. The chamber was inserted in PVC collars that were buried 5 cm deep in the soil several days before the first measurement and left in place throughout the experimental measurement period. Measurements were carried out between 8.30 am and 8.00 pm. The number of collars used varied depending on the heterogeneity of the site, with a minimum of 2 replicates per plot with at least 3 plots per site. A total of 5933 measurements were collected. Spatial replicates were averaged and then aggregated to one value per day resulting in a total of 212 days.

R_s readings were taken every 8 s during measurement periods lasting 128 s. A linear function was then fitted to increasing CO_2 concentration and time variables. The rate of soil respiration ($\mu mol\ m^{-2}\ s^{-1}$) was computed as the slope of the linear regression multiplied by a temperature calibration function (Tagesson, 2006). Data were excluded from analysis when linearity was not observed (when $r^2 < 0.9$). We assumed constant soil diffusivity at 0–30 cm.

2.3. Soil temperature measurements and Q_{10} calculation

Soil temperature (T_s) was recorded with a soil thermometer inserted in the mineral soil layer at 10 cm depth at the time of R_s measurement (in MG, CF2, OF2 and OF3 sites). In the remaining sites (CF1 and OF1), T_s was estimated using air temperature measured with the thermocouple inside the R_s chamber, subsequently applying correction functions derived from a regression model. To build this regression model we used half-hourly measurements of T_s and air temperature (EC5, Decagon Devices, Inc., Pullman, USA) from the Coruche site to build the regression model with a total of 23,497 records. The model used T_s at 10 cm depth as a dependent variable and air temperature as an independent variable. The regression analyses led to a multiple regression model with all parameters significantly different from zero ($P < 0.001$) at

Table 1
Sites description with site (ID), geographic coordinates (Lat/Long), location, dominant species (overstory/understory), years when soil respiration was measured (years), approximate tree age (age), tree density, average leaf area index of the overstory component (one side leaf area/total area of the plot), maximum aboveground biomass (AGB) in the herbaceous layer (understory max AGB), aboveground biomass in the trees (overstory AGB), leaf litterfall from the trees and C/N ratios at 0–10 cm soil depth.

ID	Lat/Long	Location	Overstory/understory	Year	Age	Tree density (trees ha^{-1})	LAI ($m^2\ m^{-2}$)	Understory max AGB ($kg\ DM\ m^{-2}$)	Overstory AGB ($kg\ DM\ m^{-2}$)	Tree litterfall ($kg\ DM\ m^{-2}\ year^{-1}$)	C/N in soil
CF1	38°38′N/8°36′W	Pegões	*Eucalyptus globulus*/C3 grasslands	2002; 2006	9	1110	2.6	0.075	13.9	0.212	13.2
CF2	38°31′N/8°01′W	Mitra	*Quercus suber*/C3 grasses	2008–2009	22	430	3.8	0.057	–	0.146	14.5
OF1	38°28′N/8°38′W	Monte Novo	*Pinus pinea*/C3 grasses	2005–2006	45	120	3.2	0.066	12.4	0.271	–
OF2	39°08′N/8°19′W	Coruche	*Quercus suber*/C3 grasses and legumes	2008–2009	~60	177	3.1	0.069	5.5	0.207	20.9
OF3	38°32′N/8°00′W	Alfarrobeira	*Quercus ilex*/C3 grasses and legumes	1999	–	55	1.6	0.179	–	0.096	11.5
MG1	38°47′N/7°25′W	Vila Viçosa	C3 grasslands with disperse Cistus sp. shrubs	2006–2007	–	–	–	0.139	–	–	–
MG2	38°36′N/8°10′W	Montemor	C3 grasslands	2008	–	–	–	0.855	–	–	16.4
MG3	38°57′N/7°12′W	Elvas	C3 grasslands	2003–2005	–	–	–	0.045	–	–	9.2

95% interval confidence and an $R^2_{adj} = 0.67$. We used this model for estimating T_s in the remaining sites admitting neglectful variations in T_s derived from soil type.

The dependency of the respiratory metabolic reactions on temperature was calculated using the Van't Hoff empirical exponential equation (Lloyd and Taylor, 1994):

$R_s = ae^{bT_s}$, where R_s is the soil respiration in $\mu mol\ m^{-2}\ s^{-1}$, T_s is the soil temperature at 10 cm depth and a and b are the model coefficients. Subsequently, the temperature sensitivity of R_s, the Q_{10}, was calculated on an annual basis for each vegetation type as $Q_{10} = e^{10b}$.

2.4. Soil moisture measurements

Soil moisture (H_s) was measured at a depth of 10 cm adjacent to the soil collars at the time of R_s measurements, using calibrated soil moisture sensors: TDR (Time Domain Reflectometry, Soil Moisture Equipment Corporation, Santa Barbara, USA) was used in CF1, OF2, MG1; Theta-probe (ML2X, Delta-T Devices, Cambridge, UK) in MG3 and PR1 profile probes (Delta-T Devices, Cambridge, UK) were used in all the other sites. Measurements of H_s were reported as volume of water per volume of soil (in %). Whenever gaps in the H_s measurements were observed, the corresponding R_s measurements were excluded from the comparative analysis of H_s with R_s or T_s.

2.5. Plant biomass, litterfall and productivity

Standing biomass per hectare was estimated by measuring all tree diameters and height in 500 m^2 stands at each site. Aboveground biomass components (leaves, trunk and branch) and leaf area index (LAI) were estimated subsequently by using allometric equations specific for each tree species (Zianis et al., 2005; Correia et al., 2010) and converted on an area basis. The LAI in OF3 includes the herbaceous layer and was estimated by a leaf area meter LICOR-2000 (Reichstein et al., 2003). Herbaceous biomass was estimated by harvesting all plant material using the quadrates method (Krebs, 1989). Tree litterfall was estimated monthly or fortnightly with litter traps located randomly in each site during a period of at least one year. The above-mentioned site variables assessment was not necessarily coincident with the time period of R_s measurements.

2.6. Carbon and nitrogen content in soils

Carbon concentration in the soil was determined by either of two methods: the dry combustion method, according to International Organization for Standardization 10694, using a CNS elemental analyser (Leco CNS-2000, Michigan) or by the wet oxidation method (De Leenheer and Van Hove, 1958). Nitrogen concentration was determined by Kjeldahl digestion analysis (Digestion System 40, Kjeltec Auto 1030 Analyser, DEcator, Sweden).

2.7. Statistical analyses

To examine the differences between vegetation cover types, or seasons of the parameters measured, we used analysis of variance (one-way ANOVA). When statistically significant differences were found, differences between group means were identified by post hoc Tukey HSD tests. When ANOVA assumptions were not fulfilled, i.e. no normal distribution of the data and/or homogeneity of variances, non-parametric tests were carried out performing a comparison on ranks, with Dunn's test being used for post hoc pairwise comparisons. The Pearson Product Moment Correlation coefficient was used to display the strength of the association

between pairs of variables. Multiple linear regressions were used to fit the T_s and the Q_{10} model. All statistical relationships were considered significant at $P < 0.05$. Statistical analyses were carried out using SigmaStat (SigmaStat for windows V 3, Dundas Software, Germany).

2.8. Modelling approach

2.8.1. Overview

Simple bioclimatic driven R_S models were selected for the present analysis. The criterion used was their previous application in similar temperate and Mediterranean-like ecosystems. Simple models have the advantage of being user friendly and the link between input variables and output is clear and easy to evaluate. The four bioclimatic models chosen are presented in Table 2. The Bayesian framework proposed by van Oijen et al. (2011) and improved by Minunno et al. (submitted for publication) with a global sensitivity analysis was used to reduce parametric uncertainty, to evaluate the models and to highlight their strengths and weaknesses. The global sensitivity analysis was carried out for the parameters and the inputs of the models, to better understand their behaviour. The models were calibrated and evaluated using a Bayesian approach (i.e. Bayesian calibration and Bayesian model comparison). The data were aggregated at a daily time step and split in two parts; half of the dataset was used for calibration and half for model comparison. The evaluation of the models was performed at a daily as well as monthly time steps.

2.8.2. Sensitivity analysis (Morris method)

Sensitivity analyses were carried out for all 4 models, using the Morris method (Morris, 1991). The Morris method is a global sensitivity analysis that evaluates the output sensitivity of a certain model to simultaneous changes in several factors. The method consists of computing basic statistics, i.e. mean (μ) and standard deviation (σ), from the distribution of a number of incremental ratios, so-called elementary effects. For more details, see Campolongo et al. (2007) and Morris (1991). The overall importance of an input factor is given by μ, and σ describes non-linear effects and interactions between factors. Campolongo et al. (2007) enhanced the Morris method by improving the sampling strategy and proposing to calculate the distribution mean of the absolute values of the elementary effects (μ^*). μ^* is calculated to solve the problem of non-monotonic models, where the effects of opposite signs could mask the importance of a factor. The factors considered in the analyses were the model's parameters (*a1*, *a2*, *a3* and *a4*) and inputs (i.e. T_s and H_s) (Table 2). The parameters ranged between the minimum and maximum values used for the Bayesian calibration (Table 2), while model input variables varied between the maximum and minimum values measured.

2.8.3. Bayesian framework

Model performance was calibrated and evaluated using the Bayesian framework proposed by van Oijen et al. (2011), consisting of model calibration, model comparison, and analysis of model-data mismatch. The Bayesian theorem states that the knowledge we have about a certain model, given the data (termed as "the posterior", $P(\theta|D)$) is proportional to the probability of the data given the model (termed as "the likelihood" $P(D|\theta)$), multiplied by the previous knowledge that we had about the model (termed as "the prior" $P(\theta)$). In mathematical terms, the Bayesian theorem is expressed through the formula:

$$P(\theta|D) = cP(D|\theta)P(\theta) \quad (1)$$

where $c = p(D)^{-1}$, with the value c being fixed.

Table 2
Soil respiration (R_s in μmol m^{-2} s^{-1}) models used in this study for the Bayesian calibration. The total annual precipitation P (mm), average annual air temperature T (°C) and land use correspond to site characteristics in which the models were developed. a_i are the parameters of the models. The parameter *a2* in M1 and the parameter *a1* in model M2, M3 and M4 are a proxy of the base respiration values, that is soil respiration under standard conditions.

	Description	Coord.	P (mm); T (°C)	Land use	*a1*	*a2*	*a3*	*a4*	Ref
M1	$a1H_s + a2$	41°24′N 1°55′E	750; 14	Coppice of *Quercus cerris*. L with 745 trees ha^{-1}	0.08–0.22	−0.22 to 0.86	–	–	Rey et al. (2002)
M2	$a1e^{a2T_s}$	41°24′N 1°55′E	750; 14	Coppice of *Quercus cerris*. L with 745 trees ha^{-1}	0.25–0.87	0.07–0.13	–	–	Rey et al. (2002)
M3	$a1e^{a2T_s}e^{a3H_s+a4H_s^2}$	36°56″N 10°22″E	559; 16	Oak–grass savanna with 194 trees ha^{-1} in California	0.0599–0.141	0.00958–0.057	13.36–28.94	−60.197 to −19.77	Tang and Baldocchi (2005)
M4	$a1e^{(-((\ln(H_s/a2)/a3)^2))}e^{(a4*(T_s-10))}$	47°07′N 5°42′E	950; 10	Mixed broadleafed mature forest with 625 trees ha^{-1} in France	2.88–3.04	0.09–0.1	0.29–0.33	1.31–1.51	Vincent et al. (2006)

The likelihood function used in this analyses was proposed by Sivia and Skilling (2006) and it is described by Eqs. (2) and (3):

$$P(D|\theta) = \prod_{i=1}^{N} \frac{1}{\sigma_i\sqrt{2\pi}} \frac{1-\exp(-R_i^2/2)}{R_i^2} \quad (2)$$

$$R_i = \frac{M_i - D_i}{\sigma_i} \quad (3)$$

where, M is the output from the model, D is the observed data and i varies between 1 and N (number of data). We used this likelihood function to better detect outliers (Sivia and Skilling, 2006), which are frequent in R_s measurements.

2.8.3.1. Model calibration. For the models *prior*, we used uniform distributions, with the minimum and maximum for H_s and T_s values based on literature reviews. These limits were expanded in order to get a higher variability that could underpin our data set. The models were calibrated using 50% of the data available randomly distributed, while the other 50% was used for model evaluation. For the Bayesian calibration (BC), Markov Chain Monte Carlo (MCMC) simulations were performed, using the Metropolis algorithm (Robert and Casella, 1999). The MCMC method aims to converge the sampling on the region of the parameter space with the highest probability density. A complete description of the Metropolis–Hastings algorithm is given in van Oijen et al. (2011). To assess a parameter's convergence of iterative simulations, the Gelman–Rubin criterion (Gelman and Rubin, 1992) was used.

2.8.3.2. Model comparison. Model evaluation was performed using Bayesian model comparison which is a powerful extension of the Bayesian Calibration, allowing evaluation of the models structure, on the basis of their relative likelihoods (Kass and Raftery, 1995; van Oijen et al., 2011). In this case the Bayesian theorem is not applied over the parameter space of a single model but over a set of models.

$$P(M_k|D) = \frac{P(D|M_k)P(M_k)}{\sum P(D|M)P(M)} \quad (4)$$

where k varies between 1 and n models. Assuming no initial preferences for either of the models ($P(\mathrm{M1}) = \ldots = P(\mathrm{Mn})$), Eq. (4) becomes:

$$P(M_k|D) = \frac{P(D|M_k)}{\sum P(D|M)} \quad (5)$$

$P(M|D)$ is the "integrated likelihood" (IL) and is defined over the whole parameter space of M, i.e. $P(D|M) = \int P(D|\theta)P(\theta)d\theta$.

2.8.3.3. Analysis of model-data mismatch. Model-data mismatch was carried out using more classical methods based on the mean squared error (MSE). MSE was decomposed in three components: bias error, variance error and phase-shift error as proposed by Kobayashi and Salam (2000).

$$\mathrm{MSE} = \overline{(M-D)^2} = (\bar{S}-\bar{D})^2 + (\sigma_M - \sigma_D)^2 + 2(\sigma_M\sigma_D)(1-r) \quad (6)$$

where M refers to model predictions and D to the observed data. Bias error gives the mismatch between simulated versus measured data, variance error describes the ability of the model to capture data variability, phase-shift error indicates if the model is able to reproduce the pattern of data fluctuation. In addition, we calculated the normalized root mean squared error (NRMSE) for each of the 4 models.

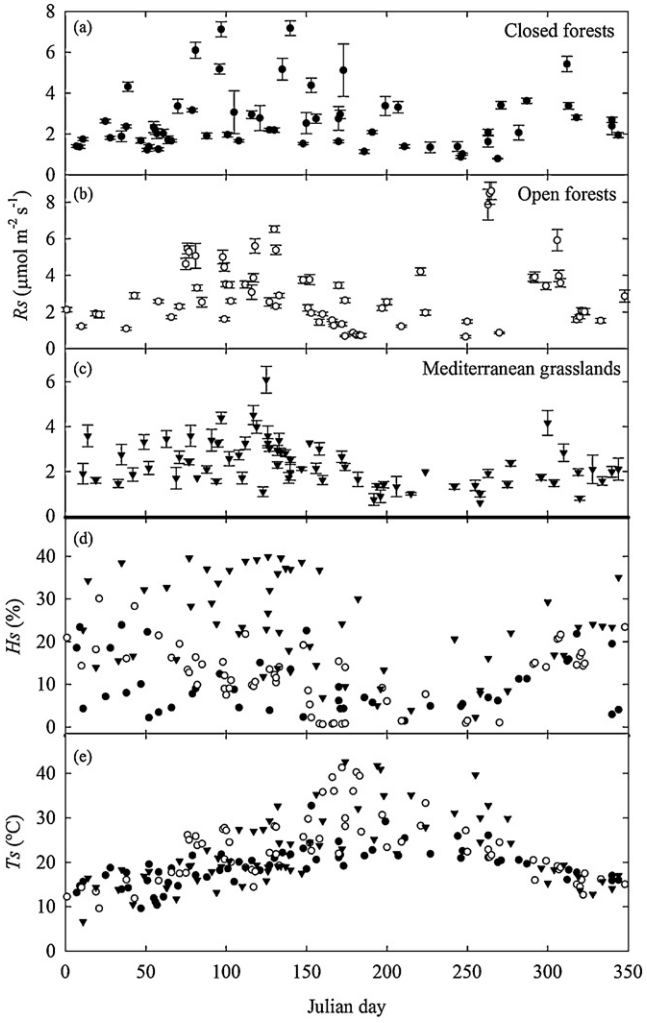

Fig. 1. Temporal dynamics of average daily soil respiration (R_s in μmol m^{-2} s^{-1}) and standard errors in the closed forest sites (a), in the open forest sites (b) and in Mediterranean grasslands sites (c); average soil water content (H_s in %) at 10 cm depth in the sites studied (d); average daily soil temperature (T_s in °C) at 10 cm depth (e) in the sites studied. Symbols: (●) closed forest, (○) open forest, (▼) Mediterranean grassland.

3. Results

3.1. Soil respiration

Seasonal R_s presented two peaks for all vegetation types: one more pronounced in spring and another one in autumn (Fig. 1a–c). Lower values were recorded in summer and in winter but no differences were observed between the three vegetation types neither on an annual ($P = 0.499$) or seasonal basis ($P = 0.222$ in autumn, $P = 0.468$ in spring, $P = 0.285$ in summer and $P = 0.150$ in winter). The highest R_s measurements were recorded in the Alfarrobeira site (OF3) in August after rain pulses with 7.9, 8.5 and 8.6 μmol m^{-2} s^{-1} (Fig. 1b). Measurement variability was highest in spring for all sites (Fig. 1a–c), and was higher in grasslands than in forested sites throughout the year.

3.2. Soil moisture and soil temperature

Similar to the fluctuations observed in R_s, soil moisture exhibited a bimodal pattern at a seasonal level following precipitation (Fig. 1d). Higher values were observed in winter and lower ones in summer. Overall, grasslands showed statistically higher H_s than

forested sites. In CF, H_s was particularly low, with many measurements below 6% in spring and summer, and values below 13% in winter.

T_s at 10 cm presented maximum values during the summer months with lower values recorded in winter (Fig. 1e) following the trend in air temperatures. Closed forests presented statistically lower soil temperatures compared with the other vegetation types. Annual soil thermal amplitude decreased with increasing canopy cover and was highest in MG (min 6.7 °C–max 42.6 °C) followed by OF (min 9.6 °C–max 41.4 °C) and CF (min 9.7 °C–max 32.8 °C) (Table 3).

3.3. Soil respiration explained by soil temperature and soil moisture

Soil moisture was the driving factor for soil respiration during most of the year (Fig. 2a–c). Soil temperature was a poor and not statistically significant predictor of R_s on an annual timescale for all vegetation types ($P = 0.620$ for CF, $P = 0.011$ for OF and $P = 0.371$ for MG).

The lowest values of R_s were obtained in very dry soils with H_s below 5%. However, a positive trend to increase with H_s was observed in all vegetation types (Fig. 2a–c). On a yearly basis and for OF sites a positive significant correlation was found between R_s and H_s (Fig. 2b). For CF sites, this correlation, although positive, was not significant (Fig. 2a). For MG, H_s alone explained 42% of the variability in R_s (Fig. 2c). T_s drove R_s response but only under certain conditions. That is, whenever soil moisture reached a certain threshold (10% for forest sites and 15% for grasslands) R_s was observed to increase with soil temperature (Fig. 2d–f). In contrast, below these limits H_s was the driving factor of R_s (Fig. 2g–i). The temperature sensitivity of R_s (Q_{10}) was therefore calculated above these limits with values of 2.01, 1.61 and 1.31 for CF, OF and MG, respectively (Fig. 2d–f).

3.4. Model performances

3.4.1. Sensitivity and uncertainty analysis

Sensitivity analysis shows how model output is affected by changes in factors (model inputs and parameters), helping to better understand model behaviour. Sensitivity results are reported in Fig. 3. Overall, models presented a higher sensitivity to the parameters than the input variables (H_s and T_s) because we used broad parameter ranges. Between the models that depend on both T_s and H_s, M3 is more sensitive to H_s and M4 to T_s (Fig. 3).

Bayesian calibration (BC) allowed reducing the uncertainty in model parameters. The uncertainty in a parameter is expressed by the variance of its distribution (Fig. 4). In this analysis, parameter uncertainty was reduced in all models as shown in Fig. 4 where the posterior marginal distributions for the parameters of each model are reported. An exception is for M4 where *a2* remained highly uncertain for CF and MG and also *a3* just for CF.

3.4.2. Bayesian model comparison (BMC)

Regarding daily measurements, the M4 model presented a higher probability of being the best model for forest sites (30% for CF and 100% for OF). For MG, the models with the best probability were M1 (55%) and M3 (45%). On a monthly basis, M1 presented the highest probability of being the best model (40%) for CF, and M4 (41%) for OF. For grasslands, M1 and M3 performed better with 34% and 32% chance of being the best model. This was also confirmed by the low NRMSE values for H_s dependent models in grasslands, especially on a monthly time step (Table 4).

In Fig. 5, observed and estimated values of R_s on a monthly time step are plotted. For each vegetation type only the best model, according to BMC, was presented (M1 for CF and Mediterranean

Table 3
Seasonal average and standard errors of soil respiration rates (R_s in μmol m^{-2} s^{-1}) in each site. Annual average and standard errors and maximum and minimum (Max–Min) values for R_s, soil water content (H_s in %) and soil temperature (T_s in °C) in each site. In brackets the number of observations in each site. (–) means not determined due to inexistent or insufficient data. R_s measurements recorded during rain pulses were excluded. *MG2 was excluded from annual averages as there are only spring measurements.

	Soil respiration (R_s)						Soil moisture (Hs)		Soil temperature (Ts)	
	Winter	Spring	Summer	Autumn	Min-Max	Year	Min-Max	Average	Min-Max	Average
CF1	1.9 ± 0.2 (10)	2.1 ± 0.2 (9)	2.0 ± 0.4 (5)	2.7 ± 0.2 (9)	1.0–3.6	2.2 ± 0.1 (33)	1.5–23.4	8.7 ± 1.4 (25)	13.9–25.5	19.1 ± 0.5 (33)
CF2	2.3 ± 0.3 (10)	3.6 ± 0.4 (8)	2.1 ± 0.6 (7)	2.7 ± 1 (4)	0.8–5.4	2.7 ± 0.3 (29)	4.3–23.9	11.4 ± 1.4 (20)	9.7–32.8	18.5 ± 1 (32)
Closed Forest	2.1 ± 0.2 (20)	2.8 ± 0.3 (17)	2.1 ± 0.4 (12)	2.7 ± 0.3 (13)	–	2.4 ± 0.2 (62)	–	9.9 ± 1.0 (45)	–	18.8 ± 0.5 (65)
OF1	1.3 ± 0.2 (3)	2.8 ± 0.3 (3)	1.7 ± 0.4 (3)	1.9 ± 0.8 (3)	0.9–3.5	2.0 ± 0.3 (12)	1.0–16.3	9.8 ± 1.9 (11)	14.4–24.6	20.3 ± 1 (12)
OF2	2.4 ± 0.2 (4)	2.8 ± 0.3 (6)	2.3 ± 0.2 (3)	–	1.6–3.8	2.5 ± 0.2 (14)	7.7–30.1	17.1 ± 1.8 (14)	9.6–33.3	21.1 ± 1.9 (14)
OF3	3.5 ± 0.5 (8)	3.2 ± 0.3 (16)	1.2 ± 0.4 (8)	2.9 ± 0.3 (9)	0.7–5.5	2.8 ± 0.2 (41)	0.7–24.6	11.8 ± 1.2 (38)	10.9–41.4	23.7 ± 1.2 (47)
Open forest	2.8 ± 0.4 (15)	3.1 ± 0.2 (25)	1.6 ± 0.3 (14)	2.6 ± 0.3 (13)	–	2.6 ± 0.2 (67)	–	12.6 ± 0.9 (63)	–	22.7 ± 0.9 (73)
MG1	2 ± 0.3 (4)	2.7 ± 0.4 (9)	1.6 ± 0.2 (9)	1.8 ± 0.2 (9)	0.8–4.5	2 ± 0.2 (31)	2.3–32.0	15.8 ± 1.6 (24)	13.2–42.6	25.1 ± 1.6 (31)
MG2	–	2.8 ± 0.2 (12)	–	–	1.6–4.0	–	30.0–39.9	37.1 ± 0.7 (14)	17.6–32.1	20.6 ± 1 (14)
MG3	2.8 ± 0.3 (8)	2.5 ± 0.4 (8)	1.1 ± 0.2 (5)	2.6 ± 0.5 (4)	0.6–4.2	2.3 ± 0.2 (25)	8.6–38.5	23.8 ± 1.8 (23)	6.7–41.0	19.5 ± 1.4 (26)
Med. Grassl.	2.5 ± 0.2 (13)	2.7 ± 0.2 (29)	1.4 ± 0.2 (15)	2.0 ± 0.2 (13)	–	2.2 ± 0.1 (56)*	–	19.7 ± 1.3 (47)*	–	22.5 ± 1.1 (57)*

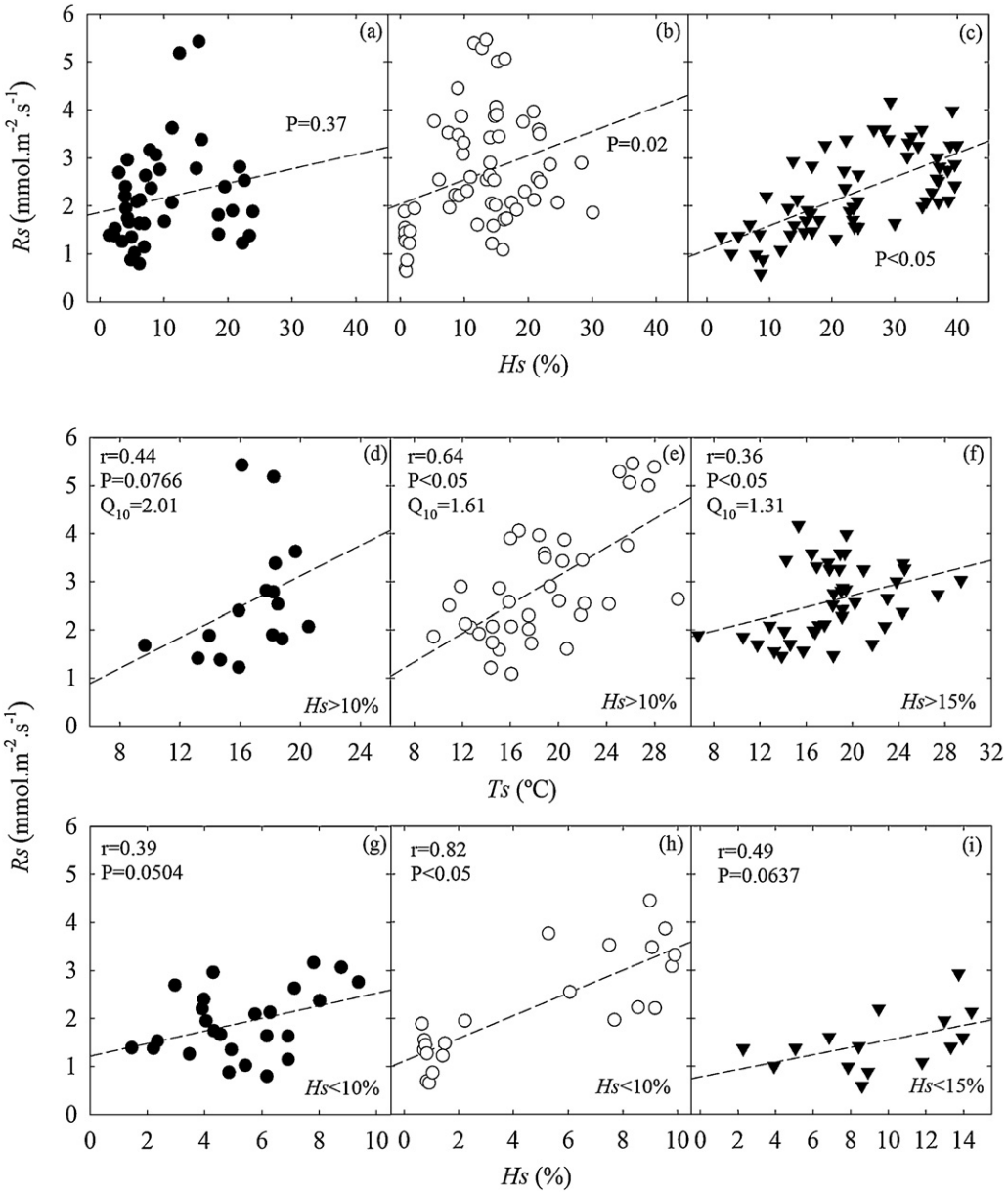

Fig. 2. Annual variation in soil respiration (R_s in μmol m^{-2} s^{-1}) correlated with soil temperature and soil water content (H_s in %) for the 3 vegetation types (a–c). Soil temperature (T_s in °C) drives soil respiration (R_s in μmol m^{-2} s^{-1}) whenever soil water content (H_s in %) is above 10% in forested sites (d and e) and above 15% in Mediterranean grasslands (f). H_s is the driving factor of R_s whenever H_s is below 10% in forested sites (g and h) and below 15% in Mediterranean grasslands (i). Symbols: (●) closed forest, (○) open forest, (▼) Mediterranean grassland.

Table 4
Model results with the Bayesian comparison. *L%* is the percentage probability of each model to be suitable to use in soil respiration estimates on a daily and monthly and time step. NRMSE is the normalized root mean squared error from the Model-data mismatch.

			M1	M2	M3	M4
Closed forests	Daily	*L%*	7.9	16.2	4.1	29.7
		NRMSE	109.8	112.7	109.9	110.2
	Monthly	*L%*	37.5	10.7	11.2	6.4
		NRMSE	125.1	153.1	93.7	120.7
Open forests	Daily	*L%*	0.0	0.0	0.0	100.0
		NRMSE	108.4	108.9	101.9	98.7
	Monthly	*L%*	8.0	5.1	30.9	40.7
		NRMSE	125.1	127.6	95.0	106.2
Mediterranean grasslands	Daily	*L%*	54.8	0.0	45.1	0.0
		NRMSE	83.8	99.7	82.2	84.1
	Monthly	*L%*	34.4	2.7	31.6	23.7
		NRMSE	47.3	83.3	44.7	47.9

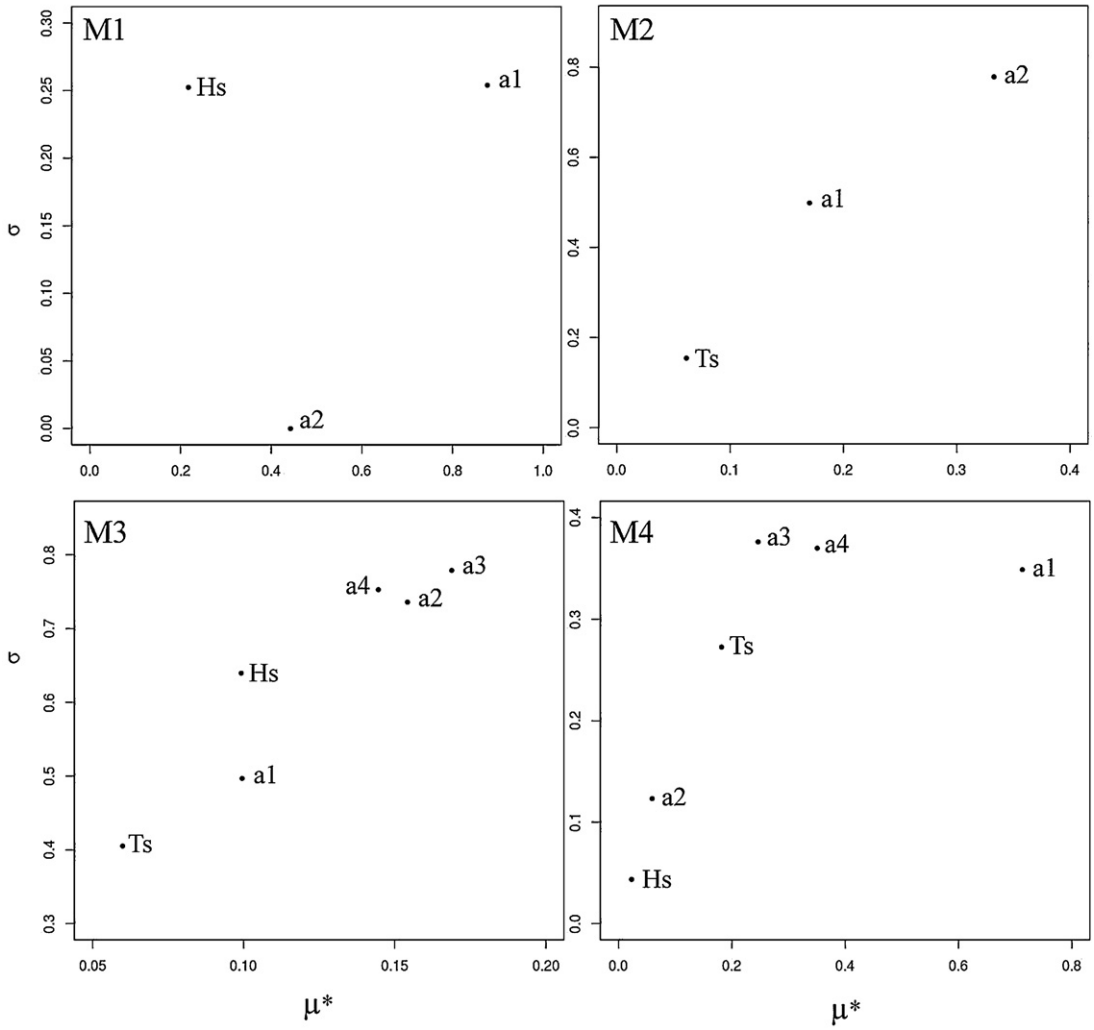

Fig. 3. Sensitivity analysis of the parameters and variables in each of the 4 models studied (M1–M4 in accordance with Table 2) where σ is the standard deviation and μ^* describes the distribution mean of the absolute values of the elementary effects. T_s is the soil temperature (in °C), H_s is the soil moisture (in %). *a1*, *a2*, *a3* and *a4* are the parameters.

grasslands and M4 for OF). The results show that M1 can be used to reliably predict R_s at monthly time steps for Mediterranean grasslands. For the forested sites, none of the models was able to accurately capture the monthly pattern of R_s and therefore they are not suitable for estimations of R_s in forest ecosystems.

3.4.3. Analysis of model-data mismatch

Results from the analysis of model-data mismatch with the decomposition of the mean square error (MSE) led to a better understanding of the models strengths and weaknesses by giving additional information on bias, variance and phase error, which was not provided by the BMC (Fig. 6). At a daily time step, none of the models were able to reliably estimate R_s. Poor performances were obtained also for both forested sites at a monthly time step. On the other hand, for grasslands, the monthly pattern of R_s was accurately predicted by the models dependent on H_s (M1) and/or T_s (M3 and M4).

For forest sites, at a daily time step, no significant differences were observed in the decomposition of the MSE. However, total MSE for OF for models M3 and M4 were slightly lower. The main component of MSE, at a daily time step, is the variance error. This means that there is a great variability in the data on a daily basis. An exception is M3 and M4 for OF, which show a higher phase error (that is a lower pattern correlation between the data).

In MG, at the daily time step, M1, M3 and M4 showed an MSE significantly lower than the other models, with the variance error being the main component for the T_s dependent model (M2), while the phase error is the highest component in M1, M3 and M4.

At a monthly time step, the differences between models performances are significant for all vegetation types. In general, the models that depend on both T_s and H_s (M3 and M4) had lower MSE, with the exception of M4 for CF. In addition, M1 had a low MSE for CF and MG. Overall the MSE was lower at a monthly compared to a daily time step. Bias error (that is the variation related to the average) was a significant component of MSE for forest sites at a monthly time step, contrasting with the very low bias error for MG sites. At a monthly time step, the variance error was very high for T_s dependent models in MG.

4. Discussion

4.1. Rates and seasonality of soil respiration

Soil respiration has two main contributing components: (i) an autotrophic component produced by roots (root carbohydrates and exudates) and rhizosphere and (ii) a heterotrophic component that result from the decomposition of organic materials by soil microorganisms (Epron et al., 1999). The relative contribution of these

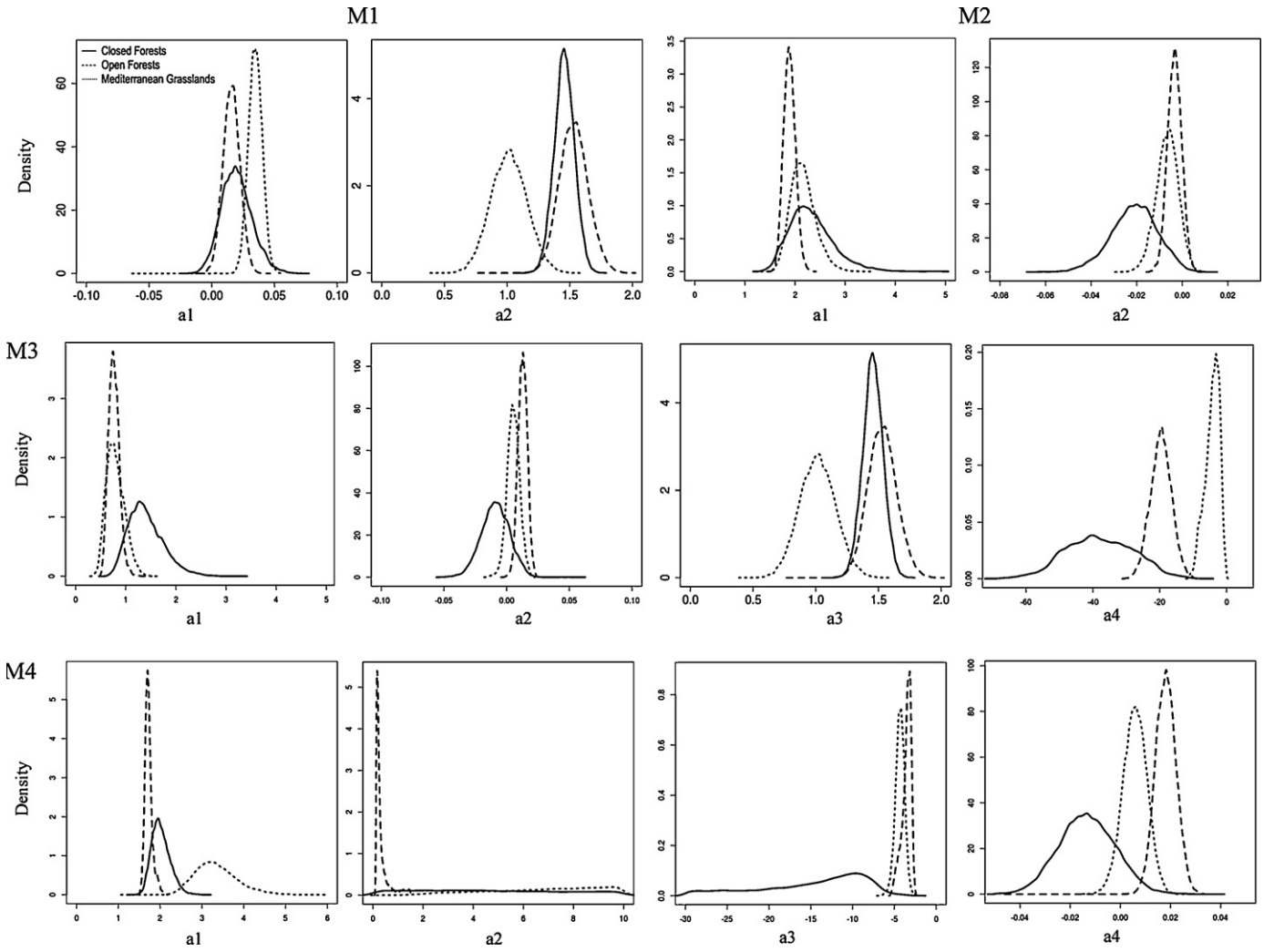

Fig. 4. Bayesian calibration results. Parameter posterior marginal distributions of the four models tested (M1–M4 in accordance with Table 2). Lines correspondence: solid line – closed forests, dashed line – open forests and dotted line – Mediterranean grasslands. Axes represent: *y* – density and *x* – range of parameters values.

components to R_s can differ along the year and between ecosystems.

The seasonality of R_s observed in the study sites followed the plant growth period and both H_s availability and the optimum temperatures for plant growth. Maximum rates also coincide with the peak of maximum photosynthesis in spring for Mediterranean ecosystems (see Fig. 5 from Pereira et al., 2007 for the seasonal variation in gross primary productivity) and also with adequate microclimatic conditions for soil microbial decomposition. For oak dominated sites (CF2, OF2 and OF3), this is also the peak of maximum leaf fall and the onset of herbaceous senescence, both events providing a higher soil organic matter availability for

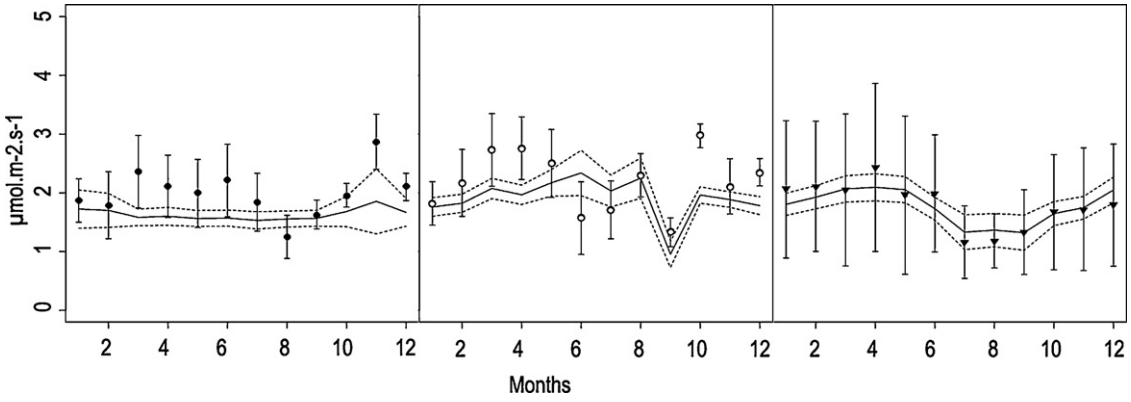

Fig. 5. Monthly observed and estimated R_s (in $\mu mol\ m^{-2}\ s^{-1}$) for each vegetation type. Estimated values represented by the solid line with upper and lower dash line as the 25 and 75% percentile, using the best model: M1 for closed forests and Mediterranean grasslands and M4 for open forests. Observed monthly averages represented by the symbols: (●) closed forests, (○) open forests, (▼) Mediterranean grassland, with the correspondent standard error in vertical bars.

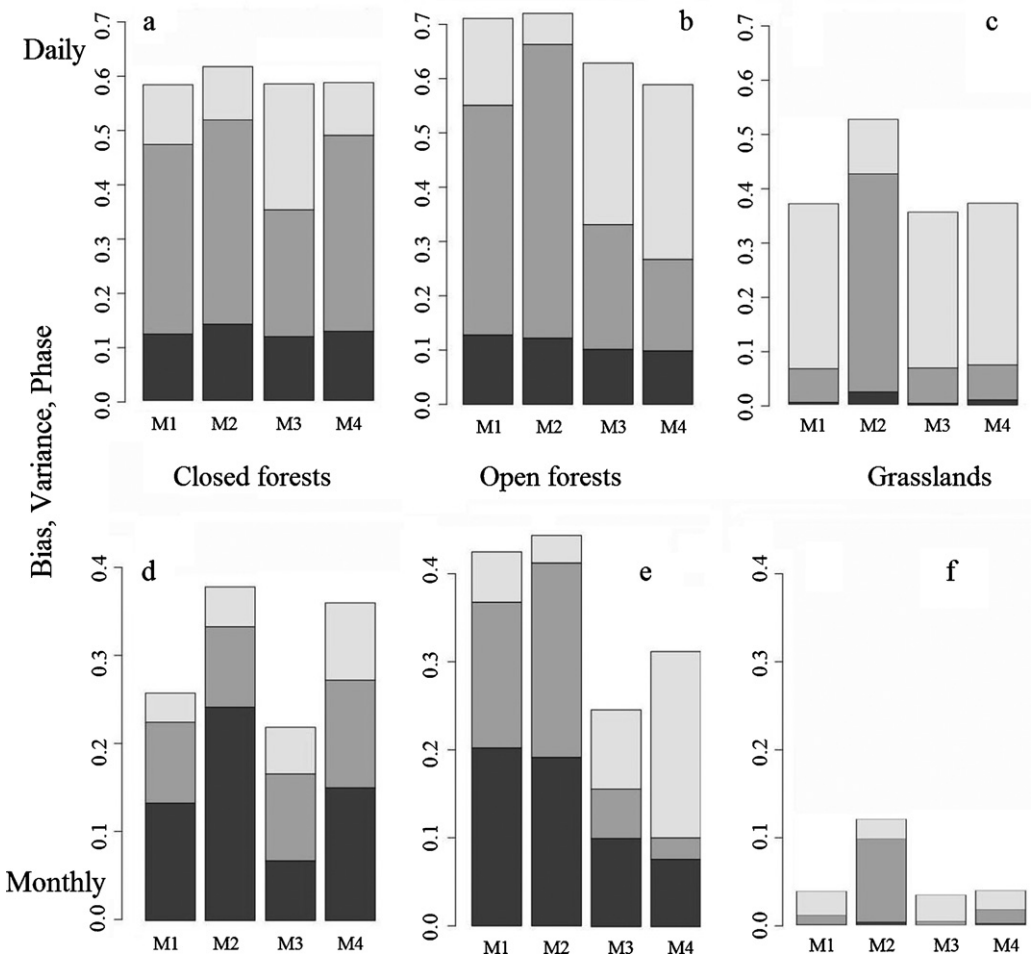

Fig. 6. Means squared error decomposition in the 3 errors: bias (dark grey), variance (medium grey), phase (light grey) and a daily and monthly time step. CF, closed forests; OF, open forests; MG, Mediterranean grasslands.

decomposition (Caritat et al., 2006). In spring, soil water is generally not limiting and higher air temperatures and radiation stimulates photosynthesis and the consequent allocation of photosynthates for new root growth (Hibbard et al., 2005). The combined effect of both autotrophic and heterotrophic respiration may account for the higher spring CO_2 effluxes.

In summer, the decrease in R_s is in accordance with the onset of the dry period followed by tree growth decline and senescence of the vegetation in annual grasslands of Mediterranean climates (Baldocchi et al., 2010). Concomitantly, water stress can limit access to labile carbon substrates and inhibit the activity of microbes in the top soil layers where the substrate for respiration is commonly abundant.

It is likely that the increase of R_s in autumn results more from a stimulation of the soil heterotrophic response and/or release of inorganic carbon from soils than from an autotrophic response, as the rain at the end of the dry season occurs before annual grassland and tree root re-growth (Pereira et al., 2007).

Although we observe a coupling between plant annual life cycle and the seasonally pattern of R_s, we do not have seasonal detailed information regarding plant productivity to provide a quantitative explanation for the similarity in R_s rates in such contrasting vegetation types. For example, in the coppiced eucalypt site (CF1), spring photosynthesis was twice that of the open evergreen woodlands (OF3) and grassland sites (Pereira et al., 2007), but this was not reflected in higher R_s suggesting that root respiration is probably a small fraction of total soil CO_2 efflux. This has been found in other studies in Mediterranean ecosystems. Unger et al. (2010), using stable carbon isotope techniques, found a root contribution to total soil CO_2 efflux of 15–28%, a much lower percentage than the estimates of up to 85% for the heterotrophic component. Others studies report values in the range of 14–29% in a Mediterranean grassland (Gavrichkova et al., 2010) and of 23% for a *Quercus cerris* forest in Italy (Rey et al., 2002).

The low contribution of root respiration to total soil CO_2 efflux may reduce the relevance of autotrophic activity in explaining R_s seasonality. However, this does not explain the effect of productivity and substrate availability related with litter decomposition in the between site variability of R_s. Thus a more detailed description of the seasonal variations in substrate availability, either through changes in productivity or transfers to decomposable pools should be included in order to increase model accuracy.

4.2. The effect of vegetation cover on soil microclimate

In our study, the similarity of R_s rates in forests and grasslands, either on an annual or seasonal time step contrasts with the findings of other studies (e.g. Hibbard et al., 2005). The higher R_s in forest ecosystems is explained by higher input of organic matter from tree litterfall, consequently stimulating heterotrophic respiration (Hibbard et al., 2005; Tedeschi et al., 2006), and the contribution of growth and maintenance respiration of tree roots (Jackson et al., 1996; Bond-Lamberty et al., 2004; Hogberg et al., 2008). However, the literature from drought prone regions on this subject is contradictory. Studies on R_s (with at least one year of R_s measurements) comparing contrasting vegetation types in semi-arid

Table 5
Average soil respiration (R_s in $\mu mol\ m^{-2}\ s^{-1}$) measurements for forests and grasslands collected in studies from the Mediterranean region.

Site	Lat/Long	P (mm)	T (°C)	R_s chamber type	R_s ($\mu mol\ m^{-2}\ s^{-1}$)	Ref
Oak–grass savanna (open areas)	36°56′N 10°22′E	556	16.3	Li-6400	0.5	Tang and Baldocchi (2005)
Degraded Steppe alpha grass	36°50′N 2°15′W	220	18	PP systems	0.8	Rey et al. (2011)
Natural Steppe alpha grass	36°56′N 2°1′W	220	18	PP systems	1.1	Rey et al. (2011)
Olive grove	38°05′N 1°47′W	370	15.5	Li-6400	1.1	Almagro et al. (2009)
Oak–grass savanna (under trees)	36°56′N 10°22′E	559	16.3	Li-6400	1.6	Tang and Baldocchi (2005)
Abandoned field with shrubs	38°05′N 1°47′W	370	15.5	Li-6400	1.7	Almagro et al. (2009)
Forest of Alleppo pine and shrubs	38°05′N 1°47′W	370	15.5	Li-6400	2.1	Almagro et al. (2009)
Holm oak forest with shrubs	41°13′N 0°55′E	658	12	PP systems	2.3	Asensio et al. (2007)
Coppiced oak chronosequence:1 year	42°24′N 11°55′E	755	14	PP systems	3.5	Tedeschi et al. (2006)
Coppiced oak chronosequence: 5 years	42°24′N 11°55′E	755	14	PP systems	4.2	Tedeschi et al. (2006)
Coppiced oak chronosequence: 17 years	42°24′N 11°55′E	755	14	PP systems	6.6	Tedeschi et al. (2006)

ecosystems report similar R_s rates between grasslands and forests (Epron et al., 1999; Tedeschi et al., 2006; Rey et al., 2011) but others not (Almagro et al., 2009) (see annual average values from these studies in Table 5). Taking into account the low contribution of root respiration in explaining within site R_s variability and the fact that there are obvious differences in substrate quality and quantity in each site (Table 1), we stress that the similarity in R_s rates may be explained by plant-mediated effects, creating soil microclimate thresholds for microbial decomposition.

First, the reduced canopy cover in grassland sites and exposure to direct sunlight warms the soil and may induce an increase in microbial decomposition (Lloyd and Taylor, 1994; Katterer et al., 1998; Yuste et al., 2007). Consistently higher T_s and H_s values were found in grasslands as compared with forests. The combined effect of higher T_s and higher H_s could probably contribute to higher values of R_s, because H_s did not limit R_s in grassland. The high leaf-fall, rapid root turnover rates and generally lower C/N in grasslands (Gavrichkova et al., 2010) may also contribute to the R_s enhancement but the data collected in these sites do not allow any statistical conclusion. Secondly, forested sites, especially CF sites, presented consistently lower H_s values (Table 3 and Fig. 1) which may be a consequence of a higher water uptake by tree roots in all soil layers and throughout the year as compared to grasslands. In this case, it is likely that the activity of microorganisms may be somewhat inhibited by the low H_s also.

In conclusion, a number of factors may be concomitantly influencing R_s and only a more detailed site-specific seasonal analysis on R_s related to plant productivity, leaf litterfall and soil substrate availability could help disentangle these responses.

The R_s average values found in this study are within the range of values obtained for other Mediterranean systems (Table 5). Although the data collected in the literature do not allow for any conclusions on the differences between grasslands and forests, a positive and significant correlation between R_s and total average annual site precipitation could be found ($r = 0.64$, $P = 0.007$) which underlines the importance of integrating water related variables in models that aim to predict annual variability of R_s in drought-prone regions.

4.3. Sensitivity of soil respiration to soil temperature and moisture

The temperature sensitivity of R_s (Q_{10}) decreased with tree cover from closed forests (2.01), to open forests (1.61) to grasslands (1.31) and was only significant above a certain H_s threshold: 10% in forests and 15% in grasslands. These soil moisture intervals are similar to the ones obtained at other sites in drought-prone regions (Davidson et al., 2006; Almagro et al., 2009). The Q_{10} values also fall within the range of values reported for other ecosystem types (Raich and Schlesinger, 1992; Kirschbaum, 1995; Jarvis et al., 2007; Almagro et al., 2009). The Q_{10} decrease from forests to grasslands is probably explained by a higher fraction of lignin compounds in forests that decomposes more slowly but is also more sensitive to increasing temperatures (Leifeld and Fuhrer, 2005). It is likely that a co-mixture of plant/soil factors may be affecting the relationship between R_s and T_s (Tedeschi et al., 2006) also at a seasonal level, as soil organic matter availability changes seasonally due to the balance between input of organic matter, stabilisation and mineralisation. From a system dynamic perspective, this would imply a site dependency of the base respiration, that is, soil respiration under standard conditions (see Table 2) with both substrate availability and quality. However, this analysis was not possible to perform because of data limitation. A more detailed approach would be to use for example, the methodology proposed by Mahecha et al. (2010) that specifically addresses the direct responses of R_s to temperature versus long-term organic matter dynamics described by the basal respiration rate.

4.4. Bayesian modelling

A comprehensive analysis of the different models was performed using the Bayesian framework, where the uncertainty in parameter and model output was significantly reduced by the calibration process. The sensitivity and uncertainty analysis provided a better understanding of model behaviour that, together with the BMC and model-data mismatch, allowed evaluating models by highlighting their weaknesses and strengths.

The models behaved differently according to vegetation type and with respect to the time step considered. In a general overview, the climatic-driven models were not suitable for R_s estimates in forested sites, either on a daily or monthly time step. As previously suggested by other authors (Reichstein et al., 2003; Migliavacca et al., 2011), there are a series of non-climatic processes driving soil CO_2 efflux in forests, e.g. plant phenology, microbial growth dynamic and soil physical processes, which cannot be captured in the over-simplistic models tested here.

On the other hand, a good performance was achieved for grassland sites, using H_s driven models on a monthly time step. This is probably caused by the straightforward correlation between grassland productivity and available water in the soil, that consequently affects the soil CO_2 efflux. Both the exploratory analysis and the Bayesian framework agreed on the relevance of H_s (with or without the integration of T_s in the model) in explaining R_s in grasslands. For example, M1 (driven by H_s) and M3 (driven by T_s and H_s) performed better than the other models. Although M4 is also driven by H_s and T_s, it performed worse than M3 because of a higher sensitivity to T_s than H_s.

In general, the model performances were worst at a daily time step, reflecting model limitations in catching the high variability of daily measurements. The improvement in models performance for a monthly time step resulted from the adjustment of the temporal scale, that reduced errors for R_s estimates and led to a smoother

seasonal pattern. Other types of errors can be attributed to equipment and different users, although in this study these errors were minimized. A more mechanistic approach is required for daily predictions of R_s for these vegetation types, but supported by background studies, e.g. on the partitioning between soil, plants and microbial components. Although there has been an increasing number of studies on this subject in the last decade (Hanson et al., 2000; Hogberg et al., 2002; Bond-Lamberty et al., 2004; Tang and Baldocchi, 2005; Tang et al., 2005; Zhou et al., 2009; Unger et al., 2010), the processes controlling soil CO_2 efflux in arid and semi-arid regions clearly need more investigation. It must be stressed that although the increase in model complexity would theoretically reproduce biological processes in a more accurate way, it will hamper the calibration processes as it increases the uncertainty associated with the parameters and variables added. Therefore a compromise must be reached between model complexity and parameter uncertainty and the research aims, including different constraint variables, as these may enable a more comprehensive evaluation of model function.

5. Conclusions

In conclusion, this study reinforces the importance of soil moisture in predicting soil respiration in drought-prone regions and should always be taken into account in upcalling exercises. Our study concluded that purely climatic driven models – dependent only on H_s or in conjunction with T_s were able to accurately predict R_s for grassland sites on a monthly time step. In contrast, forest sites presented a higher degree of variability, probably related to plant physiological processes that could not be captured by the over-simplistic models used. The high variability observed in R_s daily measurements for all vegetation types underpins the difficulty in modelling R_s at a higher temporal resolution (daily). The similarity in R_s at seasonal and annual time scales between forests and grasslands is in disagreement with other studies where such comparisons have been previously performed. This may be attributed to the effect of the vegetation on soil bioclimatic factors: forest sites presented consistently lower H_s and lower T_s that may be limiting microorgabial activity, root respiration and the net soil CO_2 efflux. The temperature sensitivity of R_s (Q_{10}) varies between vegetation types, decreasing from CF to OF to MG, stressing the need to use species specific Q_{10} in soil process models. The H_s threshold for Q_{10} also varies between vegetation types: above 10% in forested sites and 15% in grasslands. Further model improvements should be addressed in future research integrating variables or processes aimed at describing the high variability observed in R_s for forest sites.

Acknowledgements

The research was carried out with financial support from the following projects:

O Sequestro de Carbono e a Gestão Florestal Sustentável no Sul de Portugal (AGRO 8.1 I&D-543), CARBOEUROPE-IP (GOCE-CT-2003-505572), MIND (EVK2-CT-2002-00158), NITROEUROPE-IP (FP6-2004-Global3-017841), IMECC (FP6 2007-2011-026188), Effects of deer on productivity and nutrient cycling of a Mediterranean oak woodland (FCT-POCTI 63322/2004). Fundação para a Ciência e Tecnologia funded the following grants: Correia A.C. (SFRH/BD/39058/2007), Shvaleva A. (SFRH/BPD/43643/2008) and Minunno F. (SFRH/BD/65934/2009). We thank also to the following institutions for providing field work facilities: Portucel/Aliança Florestal Company (Herdade da Espirra), Fundação Casa de Bragança, Herdade de Comonte, Herdade da Machoqueira do Grou, Herdade da Mitra. The authors wish to thank the people who collaborated in field work namely Cathy Kurz-Besson, Filipe Costa e Silva, Gabriel Pita, Mário Abrantes, Mafalda Evangelista, Marta Bastos, Marta Lópes Ramos, Pedro Almeida and Raquel Lobo do Vale. We also acknowledge Peter Fay and Stephan Unger for the English review and two anonymous reviewers for the manuscript improvements.

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Sítio experimental em pinheiro manso na Herdade de Monte Novo de Palma

Câmara de respiração do solo e analisador de CO_2 *EGM-1* da marca *PP-Systems* (esquerda); Sonda PR1 da marca *Delta-T* para medição da humidade do solo (direita)

Por se encontrarem na fronteira entre o deserto e regiões temperadas, os ecossistemas mediterrânicos são particularmente importantes num contexto de alteração do clima. É fundamental entender os processos de funcionamento destes ecossistemas a variações ambientais, particularmente no que se refere ao balanço de carbono na interface solo/planta/atmosfera. Apesar do grande número de publicações científicas sobre o tema, alguns processos em ecossistemas mediterrânicos permanecem ainda por explicar. Esta dissertação contribuiu com uma humilde fracção para este conhecimento. Este trabalho produziu informação que apoia a quantificação do balanço de carbono em ecossistemas florestais de Portugal continental a vários níveis e nos seus três principais reservatórios.

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No artigo *Biomass allometry and carbon factors for a Mediterranean pine (Pinus pinea L.) in Portugal* apresentam-se modelos alométricos de biomassa e de volume, assim como factores de expansão e conversão para pinheiro manso em Portugal. Estes modelos podem ser usados em estimativas de volume de madeira e permitem quantificar o reservatório de carbono em povoamentos puros de pinheiro manso independentemente da informação de inventário que exista para esses povoamentos, quer sejam medições dendrométricas ao nível da árvore individual ou estimativas grosseiras do volume por hectare. A utilização do ajustamento simultâneo para construção dos modelos alométricos de biomassa, permitiu obter modelos mais robustos e consistentes, o que não acontece na generalidade dos modelos alométricos existentes na literatura. Com efeito, todas as equações no modelo final são ajustadas de forma simultânea de modo a que a soma das biomassas por compartimento estimadas pelos modelos parciais igualam a biomassa estimada pela equação de biomassa total. Tratando-se de uma espécie florestal ainda pouco estudada em Portugal, este estudo foi relevante pela sua aplicação imediata na produção das estimativas nacionais de biomassa de povoamentos de pinheiro manso do 5º e 6º Inventário Florestal Nacional publicado em 2010 e 2013. As equações produzidas neste artigo estão também disponíveis para consulta nestes relatórios.

Conhecer melhor o pinheiro manso, quer do ponto de vista ecológico, quer da fisiologia da espécie, é de importância estratégica para a floresta nacional. De acordo com os dados provisórios do 6º Inventário florestal nacional, as áreas arborizadas com pinheiro-manso aumentaram significativamente (+54%) nos últimos 15 anos. A reunião de um conjunto de factores dos quais se destacam o interessante na produção de fruto, a valorização do pinhão nos mercados internacionais, o sucesso de instalação das plantas no terreno e a relativa resistência a pragas e doenças, tem contribuído para o seu sucesso. Por outro lado, o interesse do proprietário florestal por esta espécie é evidente, assim como os esforços dos agentes no

sentido de organizar e dinamizar a fileira desta espécie. O impulso dado pelo "Programa de valorização da fileira da pinha/pinhão" uma iniciativa QREN, apoiada no âmbito do INATEJO tem revelado isso mesmo.

A área de pinheiro manso tem crescido fundamentalmente através da criação de novos povoamentos monoespecíficos. Uma boa parte das plantações tem acontecido a sul do Tejo, substituindo pinheiros bravos afectadas pelo nemátodo ou em consociação com o sobreiro e azinheira. Isto requer, por conseguinte, uma gestão florestal diferente da que tradicionalmente tem acontecido. A desvalorização do mercado da cortiça, fruto do declínio do sobreiro sem (ainda) soluções à vista, motiva a procura por outras espécies que confiram ao produtor uma exploração adicional, regular e intercalada com a exploração da cortiça. Será benéfica esta consociação? Poderá o sobreiro beneficiar com a presença do pinheiro manso? A gestão destes povoamentos mistos no sentido da sua sustentabilidade futura é certamente uma linha estratégica de investigação que merece atenção.

Tendo em conta que a área de pinheiro manso tenderá a aumentar num futuro próximo, releva-se prioritário investigar esta espécie no que se refere aos aspectos da fisiologia e ecologia, como por exemplo na sua resistência face a stresses ambientais. A modelação de cenários de produtividade em pinha num contexto de alteração de clima, nomeadamente no que se refere aos processos de fileira, estudos de rentabilidade e optimização dos processos de produção e transformação da pinha para produção de pinhão, são também de grande importância.

Reside uma grande incerteza sobre a contribuição dos arbustos do subcoberto no balanço de carbono em florestas mediterrânicas e não existem estudos em Portugal que tenham, até agora, quantificado este reservatório de carbono. A área de matos está a aumentar em Portugal (12% nos últimos 15 anos) sendo o abandono dos espaços rurais um dos factores que lhe está subjacente mais relevante. A ele está associado um aumento exacerbado do risco e recorrência de incêndios, que é um dos maiores obstáculos à gestão florestal nacional. Por outro lado, os arbustos mediterrânicos são espécies extraordinariamente bem adaptados às condições xerófitas do nosso clima e de regeneração face ao fogo, possuindo inúmeras vantagens competitivas de uso de recursos limitantes. Estas vantagens explicam a razão pela qual os modelos biogeográficos prescrevam este tipo de vegetação num clima futuro mais quente e seco. Apesar de grande parte dos aspectos da fisiologia e ecologia dos arbustos mediterrânicos estarem razoavelmente bem

descritos, a contribuição destes para o balanço de carbono em ecossistemas mediterrânicos, sobretudo quando em situação de subcoberto, permanece ainda por quantificar.

Os resultados do artigo *Carbon sink strength of a Mediterranean cork oak understory: how do semi-deciduous and evergreen shrubs face summer drought?* indicam que o mosaico de arbustos que evoluiu naturalmente no subcoberto de um montado na região de Coruche, é composto por espécies com estratégias de uso de luz, água e carbono contrastantes. Parte desta diferença é explicada por características morfológicas diferentes das folhas, da conformação das copas, mas acima de tudo, da forma como é explorada a água do solo pelo sistema radicular. O arbusto perenifólio (*Ulex airensis*) apresentou potenciais de água significativamente menos negativos do que as duas espécies de *Cistus* no pico do Verão. O sistema radicular profundante sugere a sua adaptação a ambientes de alguma xerofilia e uma estratégia nitidamente conservadora de uso da água. No outro extremo, o *Cistus salviifolus* apresentou uma estratégia oportunista de uso da água (que se atribuiu ao sistema radicular superficial) e da luz (atribuído à morfologia da copa), conferindo-lhe a dominância no subcoberto. As respostas das 3 espécies a um pico de chuva no Verão foram todavia, particularmente interessantes. Este pico de precipitação resultou num importante efluxo de CO_2 para a atmosfera, explicada não só pela respiração do solo, mas também pela respiração dos arbustos, particularmente dos que usam a água de forma oportunista como é o caso do *Cistus salviifolius*. Esta espécie perdeu vantagens competitivas em comparação com outras espécies com estratégias mais conservadoras (como por exemplo, o *Ulex airensis*), porque usou a água momentaneamente disponível para rehidratar as folhas velhas e expandir as pequenas folhas de Verão, que a planta não foi capaz de metabolicamente manter. Com efeito, este pico de chuva no Verão foi sucedido de várias semanas consecutivas muito quentes e secas antes das chuvas de Outono. Estes resultados permitem especular que, num cenário de mudança de climática com um aumento da variabilidade da precipitação em Verões particularmente quentes e longos, arbustos com estratégias conservadoras de uso da água sejam beneficiadas em comparação com espécies de estratégias mais oportunistas.

Estimou-se uma contribuição dos arbustos para a assimilação total do ecossistema próximo de 20%, valor bastante razoável tendo em conta que se tratam de plantas com apenas 2 anos de idade. Isto significa que a gestão dos povoamentos florestais que possibilite uma ocupação moderada do subcoberto por arbustos, poderá ser benéfica como potencial de sequestro de carbono. É importante salientar também que os matagais mediterrâneos associados ao montado, constituem habitats extremamente diversificados, por proporcionarem um mosaico de formas vegetais ricas para as espécies animais e constituem zonas preferenciais de nidificação, abrigo e alimentação. O *input* de matéria orgânica e o incremento de carbono e

azoto nos solos é também superior nestas áreas. Outros autores referem o efeito facilitador da vegetação arbustiva na sobrevivência de plantas jovens de sobreiro, com uma potencial participação no restauro destes ecossistemas. Com efeito, algumas funções dos ecossistemas florestais poderão ser valorizadas com a presença de certas formações arbustivas. Esta situação é paradigmática. Se por um lado o conhecimento empírico dita que a eliminação dos matos é essencial no controlo do risco de incêndio, por outro, parece existir hoje algum consenso científico de que, determinados níveis de ocupação arbustiva e algumas espécies, poderão ser benéficos no restauro de ecossistemas degradados como o que acontece em muitas regiões do país. Note-se que mais de 90% da região sul do país encontra-se num nível de susceptibilidade à desertificação moderado a elevado. O estrato arbóreo degrada-se, os solos carecem de água e de nutrientes. Assim, futuras investigações deveriam tentar dar resposta a questões como: Em que locais e em que circunstâncias deve ser equacionada a gestão dos cobertos arbustivos? Poderão estes auxiliar a regeneração de determinadas espécies arbóreas como o sobreiro? Que espécies arbustivas e que índices de cobertura são compatíveis com as espécies arbóreas no que se refere à competição pelos recursos ambientais?

No artigo *Soil water availability strongly modulates soil CO_2 efflux in different Mediterranean ecosystems: models calibration using the Bayesian approach* estudou-se o solo, um dos reservatórios de carbono da biosfera que mais contribui para o efluxo de CO_2 para a atmosfera. Estima-se que, em conjunto com a respiração da parte aérea, estas perdas possam ser superiores a 50% do CO_2 assimilado através da fotossíntese. Por exemplo, em montados de baixa produtividade do interior sul do país, a respiração do solo determinou se o ecossistema foi uma fonte ou sumidouro de carbono numa base anual. Conhecer a fração da respiração que é perdida pelos ecossistemas, não só nos que se refere à componente autotrófica, como à dos solos, é fundamental para a compreensão dos processos e das interações solo/planta.

Neste artigo apresentam-se resultados de 10 anos de medições de respiração de solo em ecossistemas contrastantes do sul de Portugal. Dado o elevado número de observações (cerca de 6800) a base de dados construída traduziu-se numa oportunidade de trabalhar a informação numa dimensão e escalas diferentes daquela que seria se de um único ensaio se tratasse. Aqui compararam-se medições de respiração do solo com as variáveis climáticas que melhor se correlacionam com ela: temperatura e humidade do solo por tipo de vegetação (florestas com diferentes densidade de árvores e pastagens). Concluiu-se que a humidade do solo foi sempre determinante na explicação da variabilidade observada na respiração do solo, enquanto que

a temperatura só o foi para níveis de humidade do solo acima de um determinado limite. A exploração de modelos de base empírica simples com apenas 2 variáveis (temperatura e humidade do solo) foi efectuada recorrendo à estatística Bayesiana com a vantagem de se obter uma caracterização e quantificação da origem das incertezas dos modelos. Apesar de nas florestas não ter sido possível modelar a respiração do solo com base em modelos simples, quer numa escala diária ou mensal, foi possível calibrar um modelo para pastagens mediterrânicas numa base mensal.

A água, não só a quantidade mas a sua distribuição, é um factor extraordinariamente relevante na região de clima mediterrânio e afecta não só as plantas, como já vimos anteriormente, mas também os processos que ocorrem ao nível dos solos. Negligenciar o efeito da humidade do solo na perda de CO_2 pelos solos traduz-me numa estimativa do balanço de carbono perfeitamente aleatória. A grande maioria dos modelos de base processual continuam a estimar o efluxo de carbono dos solos com base em funções simples dependentes da temperatura impossibilitando a sua aplicação para a região mediterrânica.

Assim, numa perspectivas de investigações futura, parece importante clarificar os efeitos de curta duração da humidificação dos solos, nomeadamente o *Birch effect*, sobre a actividade microbiana à superfície e em profundidade e também da alocação para as raízes de fotoassimilados recentemente produzidos como resposta da imediata disponibilidade de água do solo. Parece igualmente importante estudar a partição do carbono respirado pelas raízes e pelos microorganismos. As metodologias de avaliação desta partição não são consensuais e existe uma grande lacuna de estudos sobre o tema. Os modelos de balanço de carbono e água actualmente disponíveis na literatura e susceptíveis de serem utilizados para a região mediterrânica são manifestamente insuficientes e apresentam severas deficiências na modelação do balanço de carbono e água no solo sazonalmente. Este é provavelmente um dos tema mais prementes em termos de perspectivas de investigação futura.