



Dinàmica del carboni i el nitrogen en brolles i prats secs mediterranis després del foc

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Dinàmica del carboni i el nitrogen en brolles i prats secs mediterranis després del foc

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Pels meus pares i els meus germans...

... i per en Max

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AGRAÏMENTS

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ABREVIATURES

CIC	Capacitat d'intercanvi catiònic
COER	Carboni orgànic elemental recalcitrant
COS	Carboni orgànic del sòl
¹³ C-NMR	Tècnica d'espectroscopia per ressonància magnètica nuclear
Gt	Gigatonnes. Equival a 1000 milions de tones
MOP	Matèria orgànica particulada
MOPi	Matèria orgànica pirogènica
MOS	Matèria orgànica del sòl
NOER	Nitrogen orgànic elemental recalcitrant
PPN	Producció primària neta
TMR	Temps mig de residència



Introducció general

INTRODUCCIÓ GENERAL

L'abandó dels cultius i la colonització de la vegetació natural en el marc del canvi climàtic

Els ecosistemes forestals a nivell mundial presenten un balanç negatiu amb una pèrdua neta d'uns 5 milions ha any⁻¹ segons dades recollides durant la dècada 2000-2010. Malgrat tot, aquesta xifra ha estat un 37 % menor respecte la dècada anterior (1990-2000) (FAO 2010). L'aforestació, que consisteix en l'establiment de sistemes forestals a terres tradicionalment dedicades a un altre ús, generalment agrícola o pastoral, ha estat un dels fenòmens que ha contribuït positivament a reduir aquest balanç. Mentre que els processos de desforestació continuen donant-se, principalment a l'hemisferi sud, l'aforestació va guanyant

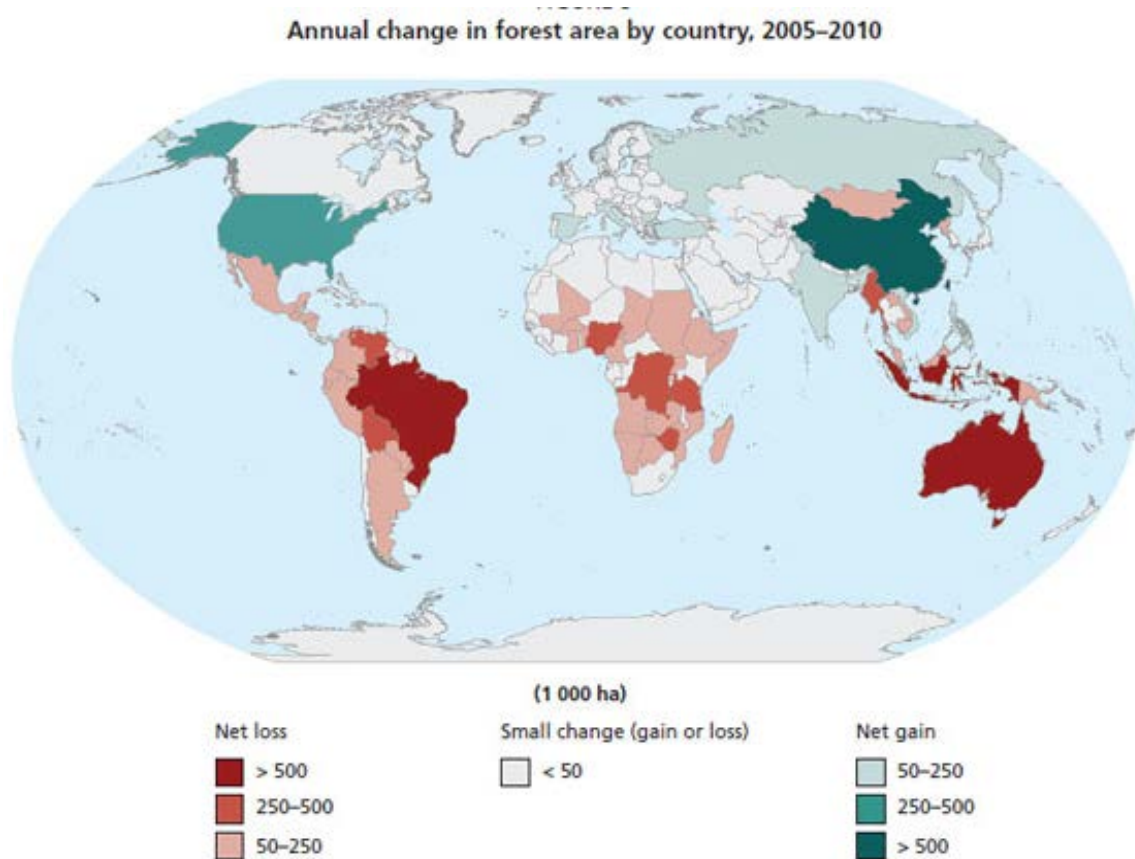


Fig. 1.1 Canvis anuals de les zones forestals en funció del país. Extret de (FAO 2010) Global Forest Resources Assessment 2010 (FAO)

terreny a l'hemisferi nord com a conseqüència de l'abandonament de les zones cultivades degut a processos d'industrialització i èxode de les zones rurals (European Environment Agency 1999) (Fig. 1.1). L'abandonament de les zones agrícoles i el procés d'aforestació han provocat un clar augment en el recobriment vegetal provocant un continu d'àrees forestals (Prieto-Fernández et al. 2004) i ha estat la principal causa de l'augment dels incendis forestals a la conca mediterrània (Moreno et al. 1998; Pausas et al. 1999). Malgrat que el nombre de focs forestals és elevat a la zona mediterrània no és massa diferent a d'altres regions d'Europa amb clima atlàntic. De tota manera, la climatologia

Average forest fire density and average burned forest fraction in Europe, 1998–2007

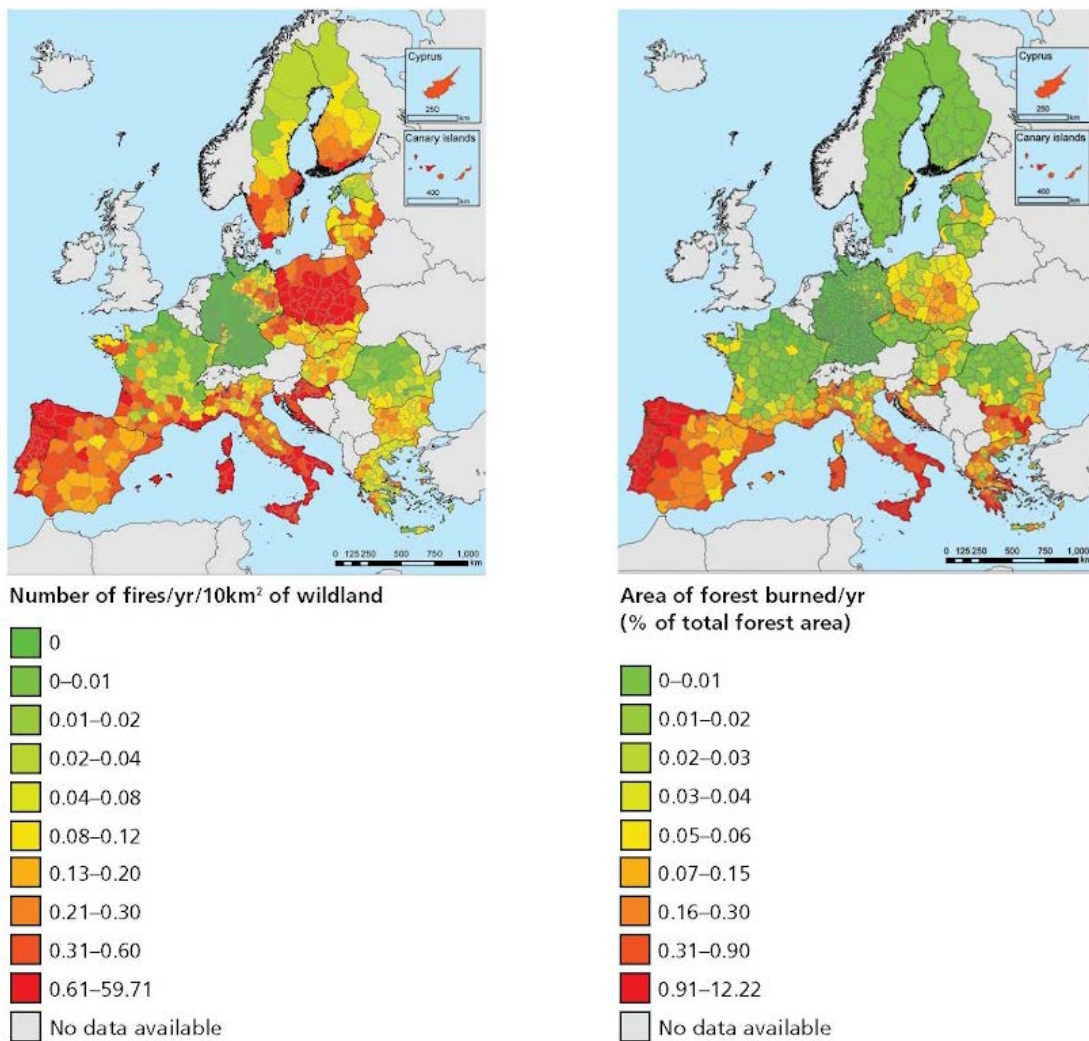


Fig. 1.2 Freqüència de focs a Europa i total d'àrea cremada degut als focs per país i per superfície de bosc. Extret de (FAO 2010) Global Forest Resources Assessment 2010 (FAO)

mediterrània fa que la propagació dels incendis i el seu efecte sobre el territori sigui molt major (Fig. 1.2). A més, les dades meteorològiques recollides durant les últimes cinc dècades han demostrat que els períodes estivals a la regió mediterrània s'han anat extremant tornant-se més secs i calorosos (Esteban-Parra et al. 2003; Pausas 2004), incrementant així la duració i la severitat dels períodes de sequera al llarg de les últimes dècades (World Bank 2003). Segons dades de l'últim informe de l'IPCC (2007) es preveu que aquesta tendència augmenti en els propers anys a resultes del canvi climàtic. Concretament, es diu que els ecosistemes mediterranis patiran un augment encara major dels períodes de sequera i, amb aquests, incrementarà el risc d'incendis forestals així com l'estrès hídric de les comunitats vegetals.(FAO 2010)

Dinàmica de la matèria orgànica i el nitrogen a cultius abandonats

La matèria orgànica del sòl (MOS) té un paper fonamental en els ecosistemes terrestres. La MOS és imprescindible pel manteniment de l'estructura del sòl, la reducció de l'erosió, la retenció d'aigua i, a més, és font de nutrients pels organismes del sòl i per les plantes. Tanmateix, en els últims anys l'estudi de la MOS ha anat prenent importància degut al seu paper com a embornal de C, ja que, a escala global, el C emmagatzemat als sòls constitueix una reserva dinàmica de magnitud major que la que es troba a l'atmosfera i a la biomassa vegetal juntes. Els canvis en l'ús del territori afecten aquestes reserves de C al sòl, de manera que, durant els dos últims segles, s'han detectat pèrdues netes de C del sòl cap a l'atmosfera degut a processos de desforestació o d'intensificació de la llaurada als camps. Per altra banda, l'augment de la conversió de les zones de conreu a zones forestals a l'hemisferi nord ha fet que l'estoc de C al sòl torni a augmentar parcialment. Als anys 90 es va descriure que aproximadament entre 2 i 4 Gt C any⁻¹ (Schimel et al. 2001) que són alliberades a l'atmosfera queden retingudes als ecosistemes terrestres. De tota manera, malgrat els esforços de la comunitat científica, el balanç global de C no s'ha arribat a tancar. En un intent de tancar aquest cicle s'ha encunyat el terme d'embornal perdut' definit com un embornal de C del que no se'n coneix el parador. Alguns autors van descriure que el misteri sobre el parador de l'embornal perdut podria ser explicat per l'augment de l'aforestació sorgida des

dels anys 50 a l'hemisferi nord (Johnson i Sharpe 1983). Contràriament, altres autors com Post i Kwon (2000) han estimat que només una petita fracció (5 - 10%) d'aquest embornal perdut podria ser explicat en termes d'acumulació de C orgànic als sòls durant processos d'aforestació. Així doncs el terme embornal perdut segueix vigent avui en dia.

Els canvis que hi pugui haver en la matèria orgànica durant l'abandó de zones cultivades depèn bàsicament de 3 paràmetres: la història dels cultius prèvia a l'abandó, la climatologia i el tipus de vegetació forestal que s'estableix després de l'abandó. Així, per exemple, Post i Kwon (2000) van trobar que els canvis en el C orgànic del sòl durant l'aforestació podien variar des de petites pèrdues en àrees temperades fredes dominades per pins fins a augments de l'orde de $300 \text{ g C m}^{-2} \text{ any}^{-1}$ a plantacions forestals subtropicals humides. En el context mediterrani, les explotacions agrícoles mil·lenàries, en alguns casos, han portat els sòls a un estat de degradació important en el moment de l'abandó. De manera que la capacitat de regeneració de les comunitats originàries per processos de successió natural, la recuperació de la qualitat dels sòls i el restabliment dels nivells de matèria orgànica originals és molt baixa, sobretot en sòls amb continguts rellevants de carbonats (Romanyà i Rovira 2011; Rovira et al. 2007). Aquest fenomen i l'efecte d'altres pertorbacions com el foc han dificultat la regeneració de boscos (als llocs on hi eren originaris) i facilitat l'establiment de prats i matollars, actualment vegetació dominant de la Península Ibèrica (Masalles i Vigo 1987).

L'aforestació afecta també a la dinàmica dels macronutrients implicats en el creixement de les plantes, com seria el cas del N. L'aforestació pot promoure la redistribució d'aquests macronutrients des del sòl cap a la formació de biomassa vegetal (Farley et al. 2008; Jobbagy i Jackson 2003). El N és un dels elements essencials que més influència té sobre els ecosistemes i sovint limita la producció primària neta (PPN). A la figura 1.3 es pot veure un esquema comparatiu del cicle del N en cultius tradicionals i en cultius abandonats. La major part de la reserva de N al sòl es troba estabilitzat a la matèria orgànica. Les capes de sòl que poden ser explotades per les arrels contenen entre 5000 i 15000 kg N/ha, però d'aquest només un 1-2% és mineralitzat i queda disponible per les plantes altra vegada (Christensen 2004). El N mineral al sòl es comporta

de diferent manera depenent de la forma que adquireixi. Mentre que el nitrat (NO_3^-) és soluble en aigua i subjecte a rentat i desnitrificació, el N en forma d'amoni (NH_4^+) és retingut al sòl mitjançant adsorció a col·loides del sòl o fixació a les argiles .

El cicle del N es veu modificat durant el procés d'abandonament. Quan el cultiu és en actiu el subministrament de N té lloc mitjançant la fertilització o a través del conreu de plantes fixadores de N_2 que puguin compensar les pèrdues de N en la collita. Si això no es duu a terme, es produeix una pèrdua gradual de N mineralitzable i, per tant, potencialment disponible per les plantes. A ecosistemes naturals, en canvi, les sortides del sistema són molt petites i el N es recicla internament directament per deposició de fullaraca i arrels o a través de les femtes dels herbívors salvatges.

El tipus de vegetació que apareix durant l'abandonament d'una zona cultivada també tindrà un paper fonamental en la dinàmica del N. Les plantes d'ecosistemes mediterranis es caracteritzen per tenir un sistema radicular molt ben desenvolupat, de manera que la biomassa radicular pot representar més del 50% de la biomassa total (Caturla et al. 2000; Sánchez-Blanco et al. 2004). Aquest sistema radicular més profund, permet d'una banda, l'absorció de N a més profunditat i d'altra banda, l'aport de matèria orgànica i exsudats de les arrels a major fondària. D'aquesta manera, el cicle del N no es limita a les capes més superficials del sòl sinó que s'amplia el rang de profunditats incrementant també la distribució de C més làbil en profunditat. D'altra banda, aquest fet pot estimular la mineralització de la matèria orgànica més antiga que ha quedat enterrada en profunditat (Fontaine et al. 2003). L'aparició de plantes fixadores de N atmosfèric després de l'abandó, pot proporcionar entrades addicionals de N a sistemes agrícoles degradats i millorar la qualitat de la MOS. A més, alguns estudis han observat que la incorporació d'espècies fixadores durant un procés d'aforestació o reforestació podia contribuir no només en una entrada extra de N al sòl si no també en un augment del segrest de C al sòl d'entre un 20 i un 100 % (Cole et al. 1995; Johnson 1992; Rhoades et al. 1998).

Cultiu en actiu

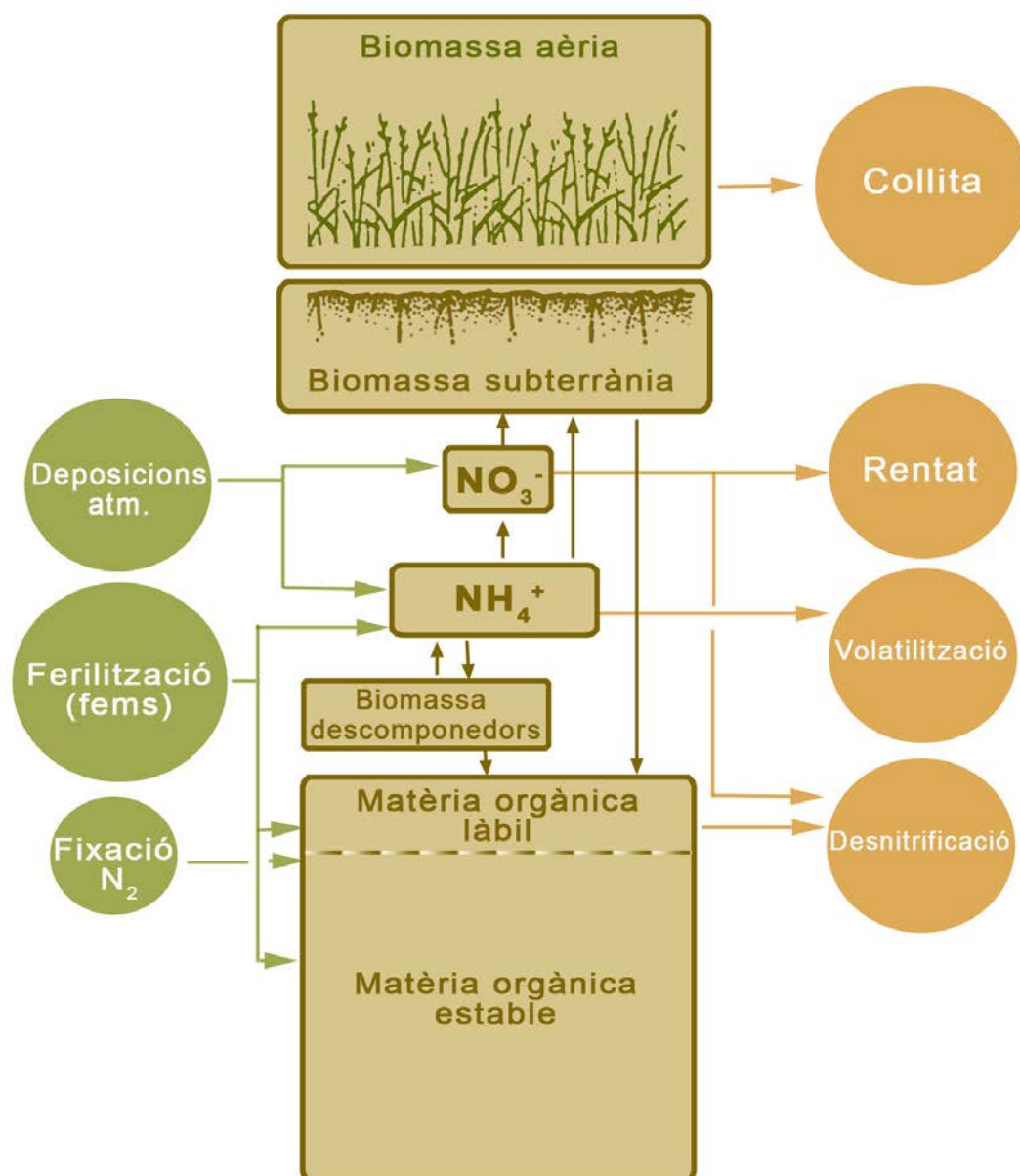


Fig 1.3a Cicle del N en un cultiu en actiu tradicional amb les diferents reserves de N i les principals entrades i sortides de N. La mida de les caixes i dels cercles mostren la relativa importància de les reserves de N i de les entrades i les sortides d'aquest element. L'esquema és una adaptació de (Christensen 2004)

Cultiu abandonat

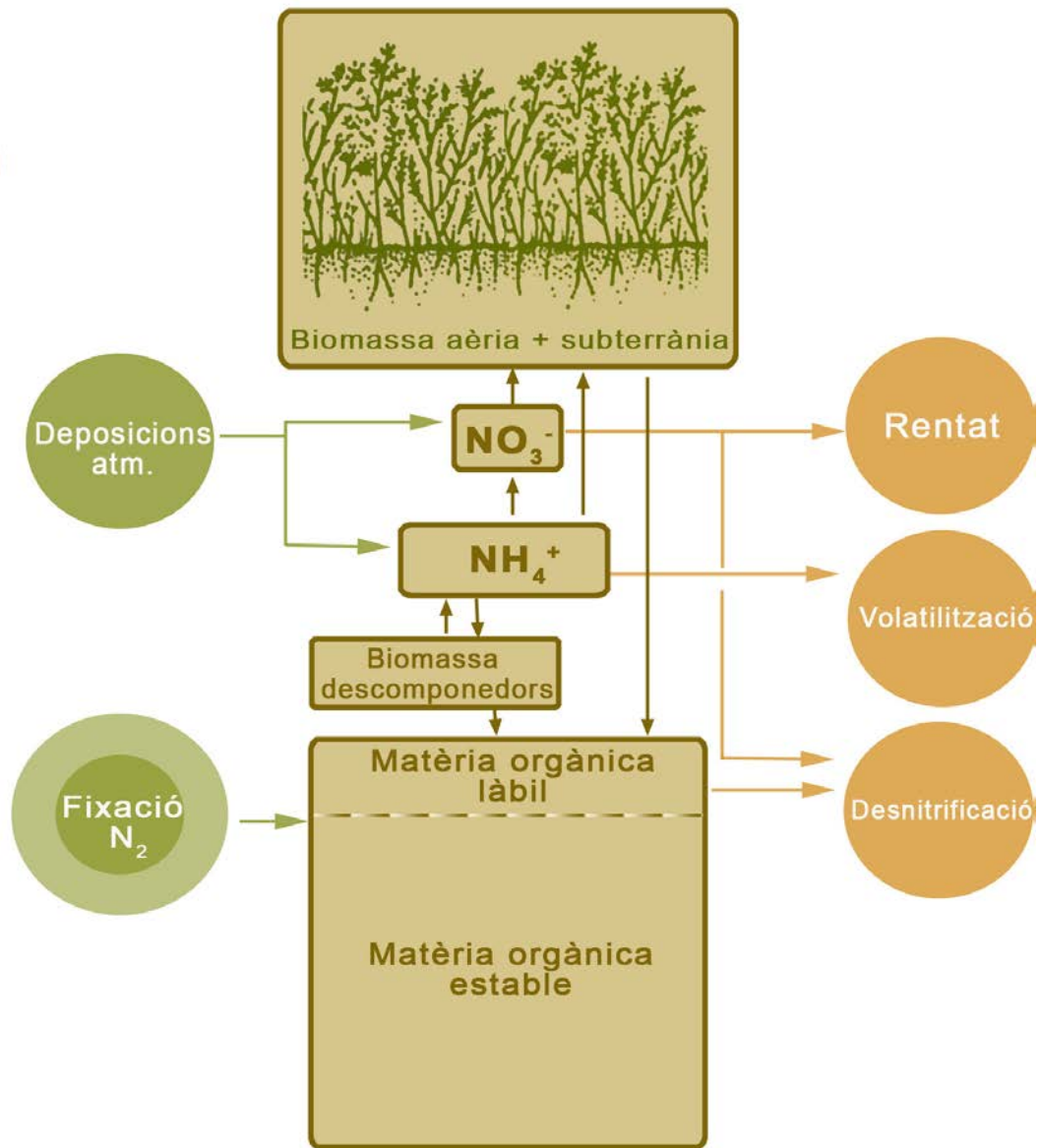


Fig 1.3b Cicle del N en un cultiu abandonat amb les diferents reserves de N i les principals entrades i sortides de N. La mida de les caixes i dels cercles mostren la relativa importància de les reserves de N i de les entrades i les sortides d'aquest element. L'esquema és una adaptació de (Christensen 2004).

Estabilització de la matèria orgànica al sòl en ecosistemes naturals

Factors que afecten l'estabilització de la matèria orgànica al sòl

L'estabilització de la MOS està determinada per un conjunt de factors organitzats jeràrquicament que operen a diferents escales temporals i espacials (Anderson i Flanagan 1989; Lavelle et al. 1992). Tradicionalment, al primer nivell de la jerarquia apareixia el clima (i.e., règims de precipitació i temperatura) considerat el major causant de variació en la descomposició a gran escala (Heal et al. 1981; Meentemeyer 1978). Al segon nivell, les característiques del material parental que influencien fortament en la textura del sòl, el pH i la riquesa de nutrients i, per últim, la qualitat de la matèria orgànica entrant que segons la seva qualitat pot dificultar o afavorir l'activitat microbiana. Sembla ser, però, que aquest sistema jeràrquic no està exempt de controvèrsia. Durant la dècada dels 90, alguns estudis realitzats a partir de llargs transectes climàtics i diferents qualitats de matèria orgànica van qüestionar l'opinió general revaloritzant la composició de la matèria orgànica com principal factor regulador de la descomposició (Berg et al. 1998; Johansson et al. 1995). Actualment, la preocupació recent davant el canvi climàtic previst per les properes dècades i el possible efecte d'aquest sobre la descomposició de part de l'embornal de C del sòl ha fet que creixés també l'interès i, amb ell la controvèrsia, entre els diferents sistemes de protecció del sòl (física i bioquímica) (Dungait et al. 2012; Krull et al. 2003). Els estudis més recents, però, aposten per una nova visió de la dinàmica del C orgànic del sòl (COS) i defensen la persistència de la MOS no tant com a conseqüència de les propietats intrínseques d'aquesta si no a les influències biològiques i físico-químiques de l'entorn edàfic que poden reduir les probabilitats de descomposició de la matèria orgànica (Dungait et al. 2012; Schmidt et al. 2011).

CONDICIONS CLIMÀTIQUES I ECOSISTÈMIQUES. Els canvis en l'estoc de C al sòl depenen del balanç entre les entrades provinents de la producció primària neta (PPN) i les sortides de C orgànic mitjançant la descomposició del COS. Geogràficament, s'ha observat que els estocs MOS estan inversament relacionats amb els gradients latitudinals de PPN, de manera que, a mesura que ens acostem a llocs més freds, les reserves de MOS augmenten i disminueixen

les entrades de matèria orgànica (Bird et al. 1996; Jones et al. 2005; Post et al. 1982). En canvi, altres regions, com per exemple les de clima mediterrani on condicions ambientals extremes s'alternen al llarg de l'any, hi són més favorables els processos de mineralització (González-Pérez et al. 2004). Així, en la majoria de models, el clima apareix com una de les eines més importants de control de la descomposició/estabilització de la matèria orgànica, essent la temperatura i la precipitació els paràmetres més freqüentment utilitzats a la majoria de models (Palosuo et al. 2012).

Les espècies vegetals que conformen una comunitat (com per exemple l'abundància relativa de plantes herbàcies o la presència de plantes fixadores de N), així com també del conjunt de canvis en l'estructura de la comunitat (cultiu, prat, matollar o bosc) són els factors que definiran la quantitat, la qualitat i la freqüència de les entrades de matèria orgànica al sòl. S'ha observat que un augment en la diversitat vegetal pot augmentar la MOS (Tilman et al. 2006), de manera que, per exemple, alguns estudis fets amb lleguminoses (fixadores de N atmosfèric) han demostrat que l'augment de la disponibilitat de N al sòl potencia l'augment del segrest de C orgànic al sòl (Kaye et al. 2000; Resh et al. 2002). A més, altres estudis van observar que tant l'aport directe de N al sòl com indirectament mitjançant l'entrada de restes vegetals riques amb N podien incrementar la descomposició de matèria orgànica recent (Berg 2000; Melillo et al. 1982) però, a la vegada, podien inhibir la descomposició del C humificat del sòl (Arnebrant et al. 1996; Berg 2000). Així Christopher i Lal (2007) va subratllar la gran importància del N com a component limitant dels processos d'humificació per la retenció de C estable al sòl.

FACTORS FÍSICS I BIOQUÍMICS. La matèria orgànica al sòl pot estar protegida a la matriu del sòl mitjançant la recalcitrància química inherent en la composició bioquímica de la pròpia matèria orgànica o bé mitjançant factors d'estabilització físics. Segons descriu (Lützow et al. 2006) aquests dos factors venen regulats mitjançant 3 processos diferents:

Preservació selectiva: Aquest procés de preservació selectiva és el que condueix a la relativa acumulació de molècules recalcitrants al sòl. Alguns autors

han definit l'existència de dos tipus de recalitrància, la primària relacionada amb la recalitrància de les restes vegetals i de les rizodeposicions i la secundària que es troba a productes formats fruit de transformacions biològiques o físiques de la matèria orgànica entrant. La matèria orgànica provinent de restes vegetals aèries o subterrànies que entra a formar part d'un ecosistema edàfic és rica en polisacàrids (midó, cel·lulosa, hemicel·lulosa i pectina; 50-60%) i lignina (15-20%) però també en proteïnes, polifenols, clorofil·la, cutina i suberina, lípids i ceres (10-20%). El grau de biodegradabilitat d'aquests compostos varia en funció de la seva composició estructural. A la vegada, la proporció relativa d'aquests components dins la planta varia en funció de l'espècie vegetal (Kögel-Knabner 2002). A mesura que aquesta matèria orgànica es va descomposant va perdent aquesta estructura simple, dificultant així la mineralització enzimàtica i convertint-se en més recalitrant (Kemmitt et al. 2008). Així doncs, la recalitrància primària és rellevant principalment durant les primeres fases de la descomposició que, en alguns casos, pot durar bastants anys. En general, la recalitrància secundària està formada per materials que provenen de restes microbianes (e.g. mureïna, quitina i alguns lípids), restes molt aromàtiques provinents d'incendis com és el carbó ('black carbon') amb temps de residència al sòl molt elevats o polímers húmics molt difícils de degradar. La qualitat de la matèria orgànica d'entrada i, per tant, la recalitrància primària pot condicionar en gran mesura la recalitrància secundària, de manera que (Berg 2000) va observar que, durant les últimes fases de descomposició de la matèria orgànica, la taxa de descomposició de residus difícils de degradar com les lignines era menor que les restes vegetals d'entrada eren riques en N i, per tant, tenien una major qualitat.

Inaccessibilitat espacial: la localització o naturalesa de la matèria orgànica sovint pot fer que microorganismes i enzims no puguin accedir a la matèria orgànica degut a la inclusió d'aquesta en agregats. Així doncs, l'oclusió té lloc, principalment, de forma biòtica de manera que la matèria orgànica particulada (MOP) queda agregada mitjançant agents cimentadors (com per exemple cèl·lules microbianes, secrecions, exsudats d'arrels, mucus de fauna) formant macroagregats (Elliott 1986; Oades 1984). L'oclusió de matèria orgànica pot ser també abiòtica, a nivell de microagregat (<20 µm), mitjançant la formació de

xarxes d'òxids de Fe i Al o hidròxids (Mayer et al. 2004). La innaccessibilitat d'organismes o enzims a la matèria orgànica també pot ser deguda a la hidrofobicitat. Aquesta pot ser causada bé per la naturalesa pròpia d'alguns compostos orgànics (lípid) o bé deguda a la unió matèria orgànica-argiles. La hidrofobicitat restringeix les condicions vitals pels microorganismes descomponedors degut a l'absència d'aigua i, consegüentment, disminueix la descomposibilitat de la matèria orgànica.

Interacció amb superfícies minerals i ions: la matèria orgànica interacciona amb superfícies minerals (principalment llims fins i argiles) dificultant-ne la mineralització i augmentant el temps de residència al sòl (Balesdent et al. 1987; Ludwig et al. 2003). Aquestes interaccions venen definides per la naturalesa química del material orgànic (com per exemple la concentració del grup funcional i la mida molecular) i el tipus de mineral de l'argila. Les associacions organo-minerals proporcionen protecció degut a l'afinitat que aquestes tenen per la matèria orgànica és molt major que la que tenen pels enzims (Dungait et al. 2012) dificultant-ne així la seva degradació. Nombrosos estudis han descrit que existeix una correlació positiva entre el contingut d'argiles (Ladd et al. 1985; Schimel et al. 1985a; Schimel et al. 1985b) i la preservació de COS, és per aquest motiu que la majoria de models inclouen la textura (%d'argiles o capacitat d'intercanvi catiònic (CIC)) com a un dels seus paràmetres que fa retardar la descomposició de la matèria orgànica, augmentant-ne el TMR de al sòl (Krull et al. 2003).

Descomposició i estabilització de la matèria orgànica al sòl. Conceptes bàsics del seu modelat

S'estima que, a nivell mundial, aproximadament unes 2344 Gt de C orgànic són emmagatzemades en els 3 primers metres de sòl. De tot aquest carboni un 54% es troba al primer metre de profunditat (Guo i Gifford 2002; Jobbágy i Jackson 2000). Això implica, que si es descomposés només un 10% del l'estoc de COS, les emissions de CO₂ a l'atmosfera equivaldrien a 30 anys d'emissions antropogèniques com les que tenim actualment i la concentració de CO₂ atmosfèric quedaria greument afectada (Kirschbaum 2000). És per aquest motiu que al llarg de les últimes dècades s'ha donat una especial importància a l'estudi

de l'estabilització de la matèria orgànica i en concret al segrest de C al sòl per contribuir a la mitigació de l'increment de CO_2 atmosfèric, principal causant del canvi climàtic mundial. La necessitat d'estudiar la dinàmica de la matèria orgànica i el segrest potencial de C al sòl a escala de paisatge ha portat precisament a l'aparició de nombrosos models que descriuen la descomposició de la fullaraca i la matèria orgànica del sòl (com per exemple el RothC, el Q, ROMUL i el Yasso07) i altres més complexos que inclouen també la producció vegetal i les seves interaccions amb el sòl (com per exemple el CENTURY, el CoupModel i el Forest-DNDC). La matèria orgànica del sòl està formada per un conjunt de restes orgàniques d'origen molt divers i amb taxes de renovació contrastades. Cada un d'aquest conjunts de restes orgàniques se l'anomena un reservori i està bàsicament caracteritzat per la seva taxa de descomposició. Precisament, la creació de models ha de servir per proporcionar prediccions fiables sobre la mida dels diferents reservoris de C en funció del tipus de sòl, vegetació, la gestió (com poden ser la llaurada, la rotació de cultius, l'aport de MOS o l'aplicació de fertilitzants) i els règims climàtics. Aquesta classe de models tenen com a finalitat predir la dinàmica de la MOS basant-se en diferents reservoris de C conceptuals diferenciats segons la seva taxa de descomposició (ràpid-actiu, lent-intermedi i molt lent/passiu/inert). Cada reservori constitueix una fracció de la MOS amb diferent estabilitat biològica o bioquímica i temps de renovació (curt, llarg, molt llarg) (Fig. 1.4). Les característiques més destacades

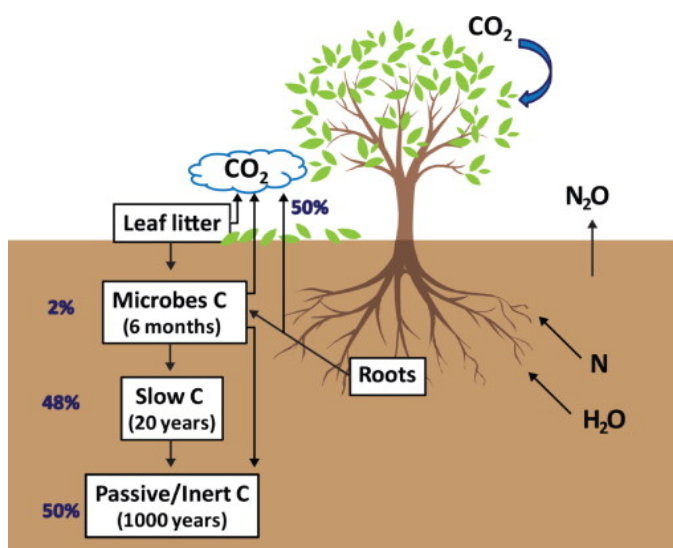


Fig. 1.4 Reservoris i fluxos conceptuals utilitzats normalment en models de MOS. Esquema extret de Stockmann et al. (2013)

que descriuen dos dels models més populars —RothC (Jenkinson i Coleman 1994) i CENTURY (Parton 1996)— es descriuen a la taula 1.1. Ambdós models divideixen la MOS en reservoris que varien intrínsecament les seves taxes de descomposició assumint que aquestes són regulades per la combinació de propietats físiques i bioquímiques. Cadascun dels reservoris té una taxa específica de descomposició i un temps mig de residència (TMR) que es veuen modificats per les condicions climàtiques. La taxa de descomposició global de la MOS és fruit de la seva distribució en fraccions i de les condicions climàtiques. Aquesta manera de fraccionar els processos de descomposició reflecteix les propietats biomoleculares i físiques concretes de cada reservori i caracteritza el seu grau d'accessibilitat a organismes descomponedors o enzims catalítics. La inclusió en els models d'aquests aspectes relacionats amb la qualitat de la matèria orgànica i la seva conjugació amb l'efecte del clima els ha conferit un potencial important tant pel que fa a la predicció dels canvis relacionats amb canvis en l'ús del territori com en la predicció dels efectes del canvi climàtic.

Així doncs, aquests models han estat aplicats amb èxit tant en ecosistemes agroforestals com en ecosistemes naturals i han estat validats en una gran varietat de climes. El seu ús a més ha permès estudiar el detall els balanços de C que tenen lloc a escala d'ecosistema.

Residue type	Century	RothC	Residence time (years)	C : N	Compounds
Litter	Metabolic	DPM	0.1–0.5	10–25	Simple sugars Amino acids Starch
	Structural		2–4	100–200	Polysaccharides
SOM	Active	BIO DPM	1–2	15–30	Living biomass POM Polysaccharides
	Slow	RPM	15–100	10–25	Lignified tissues Waxes Polyphenols
	Passive	HUM IOM	500–5000	7–10	Humic substances Clay: OM complexes Biochar

DPM, decomposable plant material; BIO, microbial biomass; RPM, resistant plant material; HUM, humified organic matter; IOM, inert organic matter; POM, particulate organic matter; OM, organic matter.

Taula 1.1 Reservoris de MOS (SOM en l'acrònim anglès) segons els models CENTURY i RothC definits d'acord amb el tipus de residu, el seu temps mig de residència (anys), la relació C:N i la classe de compostos que la formen. Taula extreta de Dungait et al. (2012)

Processos d'estabilització del nitrogen al sòl

La major part del N del sòl (95-99%) es troba formant compostos orgànics. Aquest N orgànic és poc soluble i, per tant, queda força retingut a la matriu del sòl fora de l'abast de les plantes. La majoria d'aquest N es troba en grups amino ($R-NH_2$) formant part de proteïnes (34-50%), en àcids nucleics (3-10%), en aminosucre (5-10%) o en compostos húmics la resta. El N orgànic pot ser transformat per microorganismes heteròtrofs (bactèries i fongs) i convertit en amoni (NH_4^+) (Fig. 1.5). D'aquest procés se'n diu amonificació i és el primer pas del procés de mineralització. L' NH_4^+ produït, si no és absorbit per les arrels o altres microorganismes pot ser utilitzat per bactèries autòtrofes que el convertiran a nitrat (NO_3^-) convertint-lo prèviament en nitrit (NO_2^-). Aquest doble procés és anomenat nitrificació. Els processos d'estabilització de N mineral al sòl poden ser mitjançant: (1) la retenció d' NH_4^+ a les argiles (per adsorció al complex d'intercanvi catiònic i/o per retrogradació) ; (2) la immobilització de N mineral (NH_4^+ o NO_2^-) a la matèria orgànica mitjançant processos biològics o no biològics (abiòtica).

RETENCIÓ D'AMONI A LES ARGILES. L' NH_4^+ pot ser atret per les càrregues negatives de les argiles, on pot quedar adsorbit al complex d'intercanvi catiònic. Aquest fenomen li permet estar disponible per les plantes però parcialment protegit del rentat. També, degut a la seva petita mida, pot ser retingut dins de cavitats a estructures cristal·lines de certes argiles on queda immobilitzat i fora de l'abast de la vegetació mitjançant un procés anomenat retrogradació.

IMMOBILITZACIÓ DEL NITROGEN MINERAL A LA MATÈRIA ORGÀNICA. La immobilització del nitrogen mineral a la matèria orgànica del sòl pot tenir lloc tant per processos biòtics com per abiòtics. La immobilització biòtica es realitza mitjançant la incorporació de N mineral als microorganismes amb la finalitat de formar components cel·lulars. Els mateixos microorganismes heteròtrofs encarregats de la mineralització del N orgànic necessiten N pel seu propi metabolisme i creixement, de manera que part del N mineral produït serà utilitzat per ells mateixos. Finalment, quan els organismes moren, part del N orgànic passa a formar part del complex húmic del sòl. La immobilització abiòtica té lloc mitjançant una sèrie de reaccions químiques i, tal com es mostra a la figura 4,

pot tenir lloc a partir del NO_2^- en sòls de pH àcid (≤ 5.0) o a partir del NH_3 en sòls de pH elevat (> 7.0). Rarament es troben grans quantitats de NO_2^- al sòl ja que la conversió a NO_3^- acostuma a ser un procés ràpid, però quan això passa, el NO_2^- pot reaccionar amb lignines i substàncies húmiques. Un cop el NO_2^- queda retingut a la matèria orgànica una part pot quedar fixat en aquests compostos i una altra pot evolucionar cap a la formació de N gasós (NO , N_2O o N_2). En la majoria de sòls mediterranis, de caràcter bàsic, la immobilització abiòtica de N serà mitjançant NH_3 i pot donar-se amb una gran varietat de compostos orgànics, incloent fins i tot alguns compostos de naturalesa refractària, tals com la lignina. Aquesta incorporació de NH_3 a la matèria orgànica augmenta amb

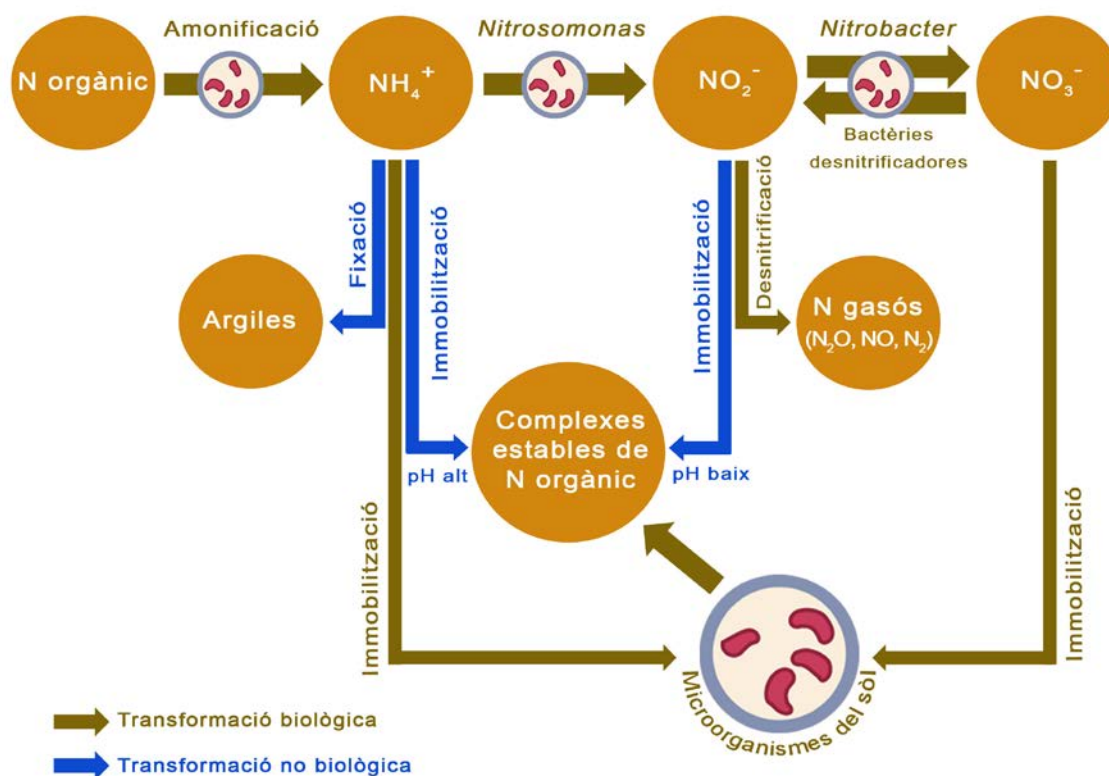


Fig.1.5 Processos biològics i no biològics d'estabilització del N al sòl. L'esquema és una adaptació de Stevenson (1982)

valors alts de pH, amb augments de MO al sòl i amb altes concentracions de NH_3 i/o NH_4^+ .

Conseqüències del foc i la sequera en la reserva de C i N del sòl

A algunes zones del món com a les regions mediterrànies on els períodes humits coincideixen amb temperatures moderades o altes, l'activitat biològica no és especialment favorable als processos d'humificació sinó a processos de mineralització. Dins aquest escenari de pèrdua progressiva de MOS, existeixen però, alguns processos que poden afavorir la formació de matèria orgànica estable al sòl com són: els incendis forestals i l'entrada de matèria orgànica degut a la deshidratació vegetal afavorida per la intensa radiació solar i dràstics cicles de sequera (González-Pérez et al. 2004).

El foc aporta grans quantitats de C i N a l'atmosfera procedents de la biomassa cremada, però també part de la necromassa s'incorpora al sòl. A més, l'augment de temperatura del sòl durant i després del foc pot provocar canvis a la MOS augmentant la presència de formes químicament més recalcitrants (Sánchez i Lázari 1999). La combustió incompleta de la matèria orgànica pot originar l'aparició de matèria orgànica pirogènica (MOPi) formada per estructures orgàniques altament condensades que li confereixen una gran resistència a la degradació. Aquesta MOPi està formada en gran part per carbó ('black carbon'), estructures aromàtiques que inclouen un ampli rang de formes: des de restes vegetals fruit de la combustió incompleta fins a carboni gràfític inert (Gélinas et al. 2001). Recentment s'ha incorporat un nou terme anomenat 'black N' (Knicker et al. 1996; Knicker 2010), fent referència a formes pirogèniques de N heterocíclic que formen part del reservori de N més recalcitrant.

Una part important del N alliberat després del foc pot quedar en forma de N mineral, principalment si es tracta de focs de baixa intensitat (Christensen 1973; Prieto-Fernández et al. 2004; Weston i Attiwill 1990). El destí d'aquest N mineral pot ser divers, de manera que pot ser rentat, assimilat per les comunitats microbianes i, per tant, immobilitzat a la reserva de N orgànic del sòl, associat a argiles o matèria orgànica per adsorció iònica o bé, utilitzat per la nova vegetació quan torni a créixer. La capacitat de retenció d'aquest N mineral al sòl és limitada i el risc de rentat i, per tant, la possible pèrdua d'aquest N soluble en cas d'episodis de fortes pluges augmenta just després de l'incendi. D'altra banda, la ràpida recuperació de l'estrat vegetal després de l'incendi també tindrà un paper

fonamental en la retenció d'aquest N mineral al sòl. L'estratègia de regeneració defineix l'ús de nutrients tan a curt com a llarg termini i, mentre que les plantes germinadores presenten una gran dependència nutricional i una ràpida eficiència en l'ús de nutrients en ecosistemes amb baixa competència, les plantes rebrotadores poden utilitzar les seves pròpies reserves de nutrients per colonitzar ràpidament nous espais (Bell i Ojeda 1999; Verdaguer i Ojeda 2002). Per altra banda, després dels focs, la presència de plantes lleguminoses pot alterar també la disponibilitat de N al sòl i millorar-ne la seva fertilitat, contribuint parcialment a compensar les pèrdues de N ocasionades pel foc (Binkley et al. 1982; Johnson et al. 2004).

Per altra banda, fenòmens meteorològics extrems, com la sequera i les onades de calor són, cada vegada més, els responsables de la mort de biomassa vegetal en ecosistemes forestals arreu del món (Fensham i Holman 1999; Lloret et al. 2004). La regió mediterrània és una de les més afectades i alguns estudis ja han mostrat els efectes negatius que la sequera provoca sobre la vegetació com l'augment en la caiguda de fullaraca (Ogaya i Peñuelas 2006; Oliveira i Peñuelas 2002) i, fins i tot, la mortalitat d'algunes espècies d'arbres quan les condicions climàtiques són extremes (Allen et al. 2010; Sarris et al. 2011). A més, les peculiars condicions climàtiques en ecosistemes mediterranis són crítiques per la descomposició de la matèria orgànica, de manera que poden limitar fortament el creixement i l'activitat microbiana (Criquet et al. 2004; Fioretto et al. 2005), provocant una acumulació de matèria orgànica al sòl. Paral·lelament, el cicle del N també es veu alterat amb llargs períodes de sequera. Larsen et al. (2011) va observar en experiments de camp amb condicions climàtiques simulades segons prediccions de canvi climàtic, que la sequera era el principal factor que afectava el cicle del N, provocant una reducció de la mineralització del N atribuït a una reducció de l'activitat microbiana.

Així doncs, les projeccions realitzades al llarg del proper segle segons l'IPCC (2007) a la regió mediterrània prediuen un augment tant en els períodes de sequera com en el risc d'incendis, així com major freqüència de períodes climàtics extrems. Els efectes d'aquests dos factors ja s'estan fent patents en l'actualitat, però els pronòstics realitzats fins ara, fan pensar que probablement els efectes sobre la biodiversitat i la dinàmica de la MOS seran importants.



Objectius



OBJECTIUS

En aquesta tesi s'ha estudiat el cicle del C i del N al llarg de 12 anys després del foc en tres comunitats vegetals típiques d'ecosistemes mediterrànies resultat de la successió que té lloc després de l'abandó de zones cultivades.

S'ha estudiat l'evolució i la recuperació del sòl post incendi en tres comunitats vegetals típicament mediterrànies que creixien en uns bancals abandonats a principis dels anys 60 del segle XX: prat de llistó, prat de llistó amb argelaga i brolla de romaní. En una primera part s'ha estudiat el C orgànic i la seva evolució al llarg de 12 anys després de l'incendi i en una segona part i, gràcies a l'aplicació de marcatge amb ^{15}N després del foc, s'ha realitzat un seguiment exhaustiu del reciclatge i estabilització del N a llarg termini en el sistema sòl planta.

Els objectius específics d'aquesta tesi han estat els següents:

- Avaluar els canvis en la reserva de C orgànic del sòl a resultes del foc i de la capacitat de resposta de la vegetació durant 12 anys en prats secs i brolles mediterrànies. (Article I)
- Determinar el destí del N mineral alliberat pel foc en el sistema sòl-planta de prats secs i brolles durant els 12 anys posteriors al foc. Es pretén veure l'efecte de la intensitat del foc, del tipus de comunitat vegetal, la presència de lleguminoses i la sequera en el cicle del N. (Article II)
- Estudiar la retenció del N alliberat després del foc al sòl i la distribució d'aquest N en diferents fraccions de matèria orgànica en funció de la mida de la partícula i la seva estabilització al llarg de 12 anys després del foc. (Article III)
- Avaluar els canvis químics ocasionats en la matèria orgànica d'origen pirogènic després del foc, determinar-ne el destí i estabilització d'aquesta al llarg de 12 anys després dels incendis i estudiar la incorporació del N mineral alliberat pel foc en aquestes fraccions. (Article IV)

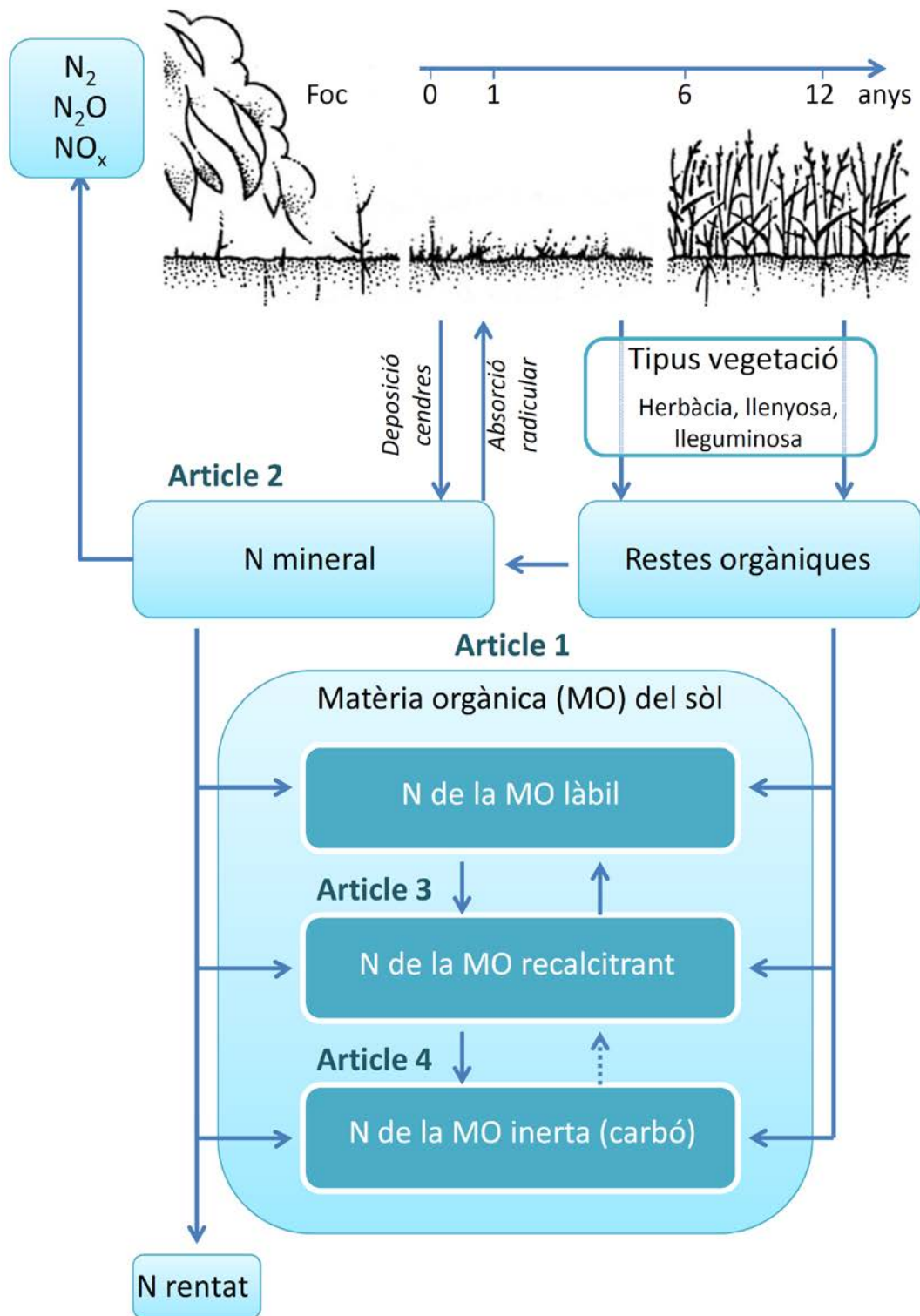


Fig. 2.1 Esquema bàsic de la dinàmica del N mineral i la matèria orgànica després del foc i els processos estudiats en aquesta tesi.



Informe del director de tesi



Barcelona, 2 de juliol de 2013

El Dr. Joan Romanyà i Socoró com a director de la Tesi titulada "Dinàmica del nitrogen en brolles i prats secs mediterranis després del foc" que ha dut a terme la doctoranda Mireia Martí Roura.

INFORMA sobre el factor d'impacte i la participació de la doctoranda en cadascun dels articles inclosos en la memòria d'aquesta Tesi Doctoral

Capítol 1. Article "**Temporal changes in soil organic C under Mediterranean shublands and grasslands: impact of fire and drought**", publicat a la revista *Plant and Soil* amb un índex d'impacte (2011) de 2.733. Aquest treball del que la Mireia Martí és la primera autora es basa en un estudi diacrònic sobre la matèria orgànica del sòl en parts secs i brolles mediterrànies després del foc. El seguiment es prolonga durant 12 anys de manera que la doctoranda només ha pogut participar físicament en els últims mostratges de camp. Per millorar la qualitat dels resultats els ha normalitzat reanalitzant les mostres de tots els sòls. A més ha calgut també fer un esforç d'organització de la base de dades ja que no en tots els mostratges es disposava del mateix patró de mostratge. Aquests aspectes han estat tinguts en compte en el tractament estadístic de les dades. La redacció de l'article ha anat també al seu càrrec. De manera embrionària aquest article va ser presentat com a pòster al congrés de internacional de 'Soil Organic Matter Dynamics: Land Use, Management and Global Change' que tingué lloc a Colorado Springs (EEUU) el juliol de 2009. És de destacar que en la realització d'aquest treball la Mireia s'ha familiaritzat amb l'ús del model de descomposició de matèria orgànica Roth-C i en la seva adaptació als ecosistemes mediterranis. Els resultats de l'article han posat de manifest la importància de l'impacte de períodes prologats de sequera i del foc sobre la dinàmica de la matèria orgànica del sòl. Aquest són aspectes de rellevància per la recerca actual en biogeoquímica de sòls en el marc del canvi climàtic. De fet en els seus dos anys de vida l'article ja ha estat citat en 5 ocasions.

Capítol 2. Article **“Long-term retention of post-fire mineral nitrogen pools in Mediterranean shrubland and grassland”** publicat a la revista *Plant and Soil* amb un índex d'impacte (2012) de 2.638. En aquest treball la Mireia ha estat la primera autora, ha realitzat les anàlisis de sòl i de planta i ha redactat l'article. El disseny de l'estudi segueix bàsicament el mateix patró que l'anterior només que en aquest cas ens centrem només en l'estudi de les parcel·les cremades. En aquestes parcel·les s'hi ha fet un seguiment del nitrogen mineral després del foc a partir d'un marcatge amb ^{15}N . El marcatge tingué lloc 3 dies després del foc en el mateixos prats secs i brolles del capítol anterior. Per la realització del treball ha calgut familiaritzar-se amb els mètodes de càlcul per quantificar el seguiment del marcatge. En aquest treball s'ha vist que una bona part del nitrogen procedent de la reserva mineral de després del foc ha romàs en el sòl durant més d'una dècada, sobretot en les vegetacions sense mates fixadores de nitrogen. Aquest fet posa de manifest el grau d'adaptació de la vegetació mediterrània al foc consistent en un estalvi de l'ús del N malgrat ser uns ecosistemes de baixa productivitat. També posa de manifest el fet que la vegetació amb lleguminoses no utilitza el nitrogen del sòl d'una manera tant eficient. De moment l'article s'ha publicat a una revista de gran difusió però a hores d'ara només s'ha publicat *on line* i per tant encara no ha estat citat.

Capítol 3. Article **“Post-fire mineral nitrogen allocation and stabilisation into soil particle size fractions in Mediterranean grassland and shrubland”** No publicat. La versió presentada a la tesi constitueix la versió revisada i consensuada pels diversos autors per ser presentada a la revista *Soil Biology and Biochemistry*. La Mireia ha seleccionat i analitzat totes les mostres, ha fet el tractament de dades i ha redactat i corregit el manuscrit incloent els comentaris dels diversos autors. En aquest treball es pretén estudiar en quines fraccions granulomètriques s'estabilitza el N procedent de la reserva mineral de després del foc i veure fins a quin punt aquesta estabilització és diferent segons la mida de les partícules a les que s'associa la matèria orgànica del sòl. A partir d'aquesta base de dades s'ha pogut discutir sobre els mecanismes

d'estabilització del N al sòl i també sobre el seu grau d'estabilitat posant un èmfasi especial a les diferències observades segons el tipus de vegetació. Hem vist que el N retingut als sòls sota mates lleguminoses s'ha estabilitzat més que en els altres casos. Pensem que aquest tema té importància a l'hora de predir els efectes de la qualitat inicial del substrat en la acumulació de nitrogen i de matèria orgànica al sòl a llarg termini.

Capítol 4. Article "**Black C and black N dynamics after fire in Mediterranean grassland and shubland**" No publicat. La versió presentada a la tesi constitueix una versió revisada y consensuada pels seus autors per ser presentada a la revista *Soil Biology and Biochemistry*. La Mireia ha seleccionat i analitzat totes les mostres, ha fet el tractament de dades i ha redactat i corregit el manuscrit. També s'ha familiaritzat amb l'ús de la ressonància magnètica nuclear (RMN) per determinar la composició del C residual després d'un atac prolongat amb dicromat i amb àcid sulfúric a temperatura ambient. Aquest carboni residual es creu que representa la fracció carbonitzada o pirogènica de la matèria orgànica (black carbon) que es forma com a resultat dels focs que hi puguin haver hagut en un sòl determinat al llarg del anys. En aquest article hem volgut estudiar la dinàmica d'aquesta fracció carbonitzada en el marc experimental que ja hem explicat en els altres capítols. Per l'estudi d'aquesta fracció no hi ha una metodologia ben establerta. D'entre els mètodes existents a la bibliografia n'hem seleccionat un amb criteri d'evitar canvis en la configuració de la matèria orgànica associats a l'escalfament. A partir d'aquest mètode hem estudiat els canvis d'aquesta fracció com a resultat del foc i al llarg del temps. També hem pogut fer un seguiment de la incorporació i de la dinàmica del N en aquesta fracció. Hem vist que aquesta fracció altament resistent a l'oxidació estava formada sobretot per compostos alifàtics hidrofòbics i que el N que s'hi incorpora pot ser remobilitzat a taxes baixes. Els nostres resultats posen de manifest que aquesta fracció no es correspon gaire amb la fracció pirogènica resultant del foc sinó que la seva dinàmica es veu afectada també per l'entrada de matèria orgànica que es dona en condicions fisiològiques de l'ecosistema. Aquests

resultats poden tenir una rellevància especial a l'hora d'encarar futurs estudis sobre canvis en la matèria orgànica molt recalcitrant i sobre la matèria pirogènica al sòl.





Resultats



Capítol 1:

Canvis temporals en el C orgànic del sòl sota matollars i prats
mediterranis: impacte del foc i la sequera

Chapter 1:

Temporal changes in soil organic C under Mediterranean shrublands and
grasslands: impact of fire and drought

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Resum del capítol 1

Les zones forestals han augmentat a la conca mediterrània al llarg de les últimes dues dècades degut a l'abandonament de l'agricultura. Això i l'aparició d'intensos períodes de sequera han conduït a un increment en la freqüència i intensitat dels focs. Incendis i episodis de sequera poden augmentar l'acumulació a curt termini de C orgànic al sòl com a resultat de l'augment de residus de plantes. En aquest estudi, vam examinar els canvis en el C orgànic del sòl i els efectes del foc i la sequera durant un període de 12 anys en dos prats i un matollar mediterranis. D'aquesta manera, vam establir 6 parcel·les per cadascuna dels tres tipus de vegetació i vam realitzar 18 focs experimentals. Els sòls van ser mostrejats 3 dies, 9 mesos, 6 anys i 12 anys després dels focs i s'hi va analitzar el C orgànic. Vam utilitzar el model RothC-26.3 per ajudar-nos a interpretar els canvis observats. Tres dies després del foc, la quantitat de C orgànic va ser major a les parcel·les cremades que a les no cremades en els primers 5 cm de sòl. Això va ser així a les comunitats vegetals estudiades i va ser probablement degut a la deposició de plantes cremades després del foc. En canvi, aquestes diferències van desaparèixer als següents anys. En alguns casos, el C orgànic de les parcel·les cremades i no cremades van mostrar un gran increment entre els anys 6 i 12, que coincidí amb un extens període de sequera que va durar 4 anys. Els nostres resultats van indicar que a matollars i prats emmatats mediterranis la influència de la sequera la influència de períodes de sequera podria produir pics transitoris de C que són molt més grans que els pics produïts pel foc. Els pics de C causats per la sequera haurien de ser considerats durant l'estudi de la dinàmica del C orgànic del sòl en el marc del canvi climàtic global.

Temporal changes in soil organic C under Mediterranean shrublands and grasslands: impact of fire and drought

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Abstract Forest areas have increased in the Mediterranean basin over the last two decades, due to the abandonment of agriculture. This and the occurrence of intense drought periods have led to an increase in the frequency and intensity of fires. Fire and drought can increase short-term soil organic C accumulation as a result of increased plant residues. In this study, we examined the changes in the soil organic C and the effects of fire and drought during a 12-year period in two Mediterranean grasslands and a shrubland. Thus, we established 6 plots for each of the three vegetation type and we set 18 experimental fires. Soils were sampled 3 days, 9 months, 6 years and 12 years after the fires and were analyzed for organic C. We used the RothC-26.3 model to help interpret

the changes we observed. Three days after the fire, the amount of organic C was higher in burned plots than in unburned plots down to a depth of 5 cm. This was true in all plant communities under study and was probably due to burned plant deposition after the fires. However, these differences disappeared in the following years. In some cases, organic C from burned and unburned plots showed a large increase between years 6 and 12, which coincided with an extended 4-year drought period. Our results indicate that in Mediterranean shrublands and mixed shrub-grasslands the influence of drought periods could produce transient pulses of C that are much larger than the pulses produced by fire. The pulses of C caused by drought should be considered when studying the soil organic C dynamics in the frame of global warming.

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Model RothC-26.3

Introduction

During recent decades, the Mediterranean basin has undergone major changes in land use and landscape. The abandonment of agriculture has promoted increases in forest cover, leading to large continuous areas of vegetation (Moreira et al. 2001; Prieto-Fernández et al.

2004). Thus, changes in land use since the 1960s are considered the main factor in the exponential increase in the number and extension of fires (Moreno et al. 1998; Pausas and Vallejo 1999). At the same time, there is evidence that summers in the Mediterranean region have become warmer and drier over the last five decades (Esteban-Parra et al. 2003; Lebourgeois et al. 2001; Pausas 2004; Piñol et al. 1998). Consequently, the IPCC report (2007) predicts an increase in the wildfire risk and drought incidence in Mediterranean ecosystems.

There are many studies on how fire affects the total amount of organic matter in forest ecosystems. Fires release large amounts of carbon (as CO₂, CO and CH₄) and nitrogen (as NO_x and N₂) to the atmosphere whereas considerable amounts of partly charred necromass incorporates into the soil. Soil properties can experience short-term, long-term or permanent fire-induced changes, depending on the severity and frequency of fires, and the post-fire climatic conditions (Certini 2005). Plant regeneration dynamics can also affect post-fire soil properties. Several factors such as fire type, intensity, and even slope position influence the fate of the organic matter immediately after fires (González-Pérez et al. 2004). Thus, fire can cause losses of organic matter, as observed by Fernández et al. (1997), higher than 50% in the upper 10 cm of a burned pine forest or increases in organic matter due to deposition of dry leaves and charred plant materials (Almendros et al. 1990; Chandler et al. 1983; Knicker et al. 2005). In some cases, fire-induced organic C increases in the A horizon have persisted for more than 10 years after fire (Johnson and Curtis 2001). The recovery of soil organic matter in the burned areas starts with the regrowth of vegetation and is generally fast, thanks to the high net primary productivity of secondary ecological successions (Certini 2005). Indeed, net primary productivity of the secondary succession may determine the long-term changes in soil organic C after fire (Duguy et al. 2007).

In addition to fire, drought is an important factor in Mediterranean ecosystems and it can also have relevant effects on soil organic C. As water stress is one of the main constraints in Mediterranean ecosystems, drought will probably be one of the consequences of climate change with the greatest impact on this area (IPCC 2007). Drought leads to limited plant growth (Bréda et al. 2006; Peñuelas et al. 2007).

Furthermore, it often enhances litter fall (Harley et al. 1987; Ogaya and Peñuelas 2006; Oliveira and Peñuelas 2002; Pesoli et al. 2003) as a result of increased senescence. In contrast, litter fall decreases as a result of drought have also been suggested (Peñuelas et al. 2007). Low soil water availability promotes changes in plant physiology and, in cases of extreme soil water depletion, individual survival may also become critical (Bréda et al. 2006). Although root studies are scarce, extensive root mortality as a consequence of severe drought has been documented in various ecosystems (Bloomfield et al. 1996; Lloret et al. 1999; López et al. 1998; Padilla and Puignaire 2007). In dry climates, plants may allocate resources to deep roots in the search for water. In the long term, this should increase root biomass and turnover in deep layers.

In this study, we hypothesize that drought and fire will produce significant changes in the soil organic matter content of Mediterranean ecosystems. Plant communities with low above-ground biomass are expected to be less sensitive to drought and produce low intensity fires, while communities with higher biomass will produce fires of higher intensity and may be more sensitive to drought. The reduced biomass of plants that regrow after fire may be viewed as a trade off to cope with water stress during drought.

The aim of this study was to determine over a 12-year period the changes in soil organic C stocks and the effects of fire and drought on three burned and unburned plant communities growing on abandoned fields in the Mediterranean region.

Material and methods

Study site

The study was carried out in a set of abandoned fields located in the northeastern Iberian Peninsula (41° 56' N, 0° 37' E, 460 m a.s.l.). The climate is dry Mediterranean continental, the mean annual temperature is 13.5°C and the mean annual precipitation is 516.9 mm (observation period: 1996–2008; Monestir de les Avellanes, 41° 52' N, 0° 45' E, 580 m a.s.l.). The rainfall distribution is markedly seasonal, with maximum values in spring and autumn and a dry season that usually lasts from June to September.

The soils are Calcaric Cambisol (FAO-UNESCO 1988), developed from a fine textured Eocene limestone and marl colluvium, with a pH (H₂O) of 8.2 and 60% calcium carbonate content. The slope was modified by terracing and the fields were abandoned in the early 1960s. At present, the old fields are formed by three plant communities that are widely distributed in the Mediterranean basin: grassland, mixed shrub-grassland and shrubland. The grassland is dominated by the perennial resprouting grass *Brachypodium retusum* (Pers.) Beauv. In mixed shrub-grassland, the leguminous shrub, *Genista scorpius* L. in Lam et DC is scattered over a grassy sward of *B. retusum*. The shrubland is dominated by the evergreen obligate seeder, *Rosmarinus officinalis* L. The study areas had been free of fire and grazing by domestic animals for at least 10 years before the beginning of the study.

Climate

During the experiment period (1996–2008), we obtained annual rainfall and mean annual temperature data from the nearest meteorological station, which is around 10 km from the plots (Table 1). The lowest rainfall values were found from 2004 to 2007, and the warmest year studied was 2008. Average monthly and annual potential evapotranspiration (PET) was calculated with the Thornthwaite formula (Thornthwaite and Mather 1957):

$$PET = 16 \left(\frac{10 T_m}{I} \right)^a \quad (1)$$

$$I = \sum \left(\frac{T_m}{5} \right)^{1.51} \quad (2)$$

$$a = (67.5 \times 10^{-8} I^3) - (77.1 \times 10^{-6} I^2) + (0.0179 I) + (0.492) \quad (3)$$

PET is the potential evapotranspiration in mm, T_m is the average monthly temperature (°C) and a is an exponent derived from the heat index (I).

Annual water deficit (WD) was calculated using the equations described by Thornthwaite and Mather (1957). WD values were estimated assuming 100 mm of available water-holding capacity and using the Thornthwaite PET estimates.

Experimental design and soil sampling

Six pairs of plots (from 20 to 60 m²) of each plant community were established. Each pair of plots was distributed in different terraces. In 1996, one randomly selected plot of each pair was burned. The other pair remains as unburned control plot and represented vegetation and soil in an undisturbed state. Thus, 12 experimental fires were set in the grassland and mixed shrub-grassland plots in October 1996; and 21 days later, in November, six shrubland plots were burned after cutting all the shrubs and letting them dry for a week. Fire intensity was low to medium, with temperatures at ground level from 300 to 526°C. See Romanyà et al. (2001) for more details.

A soil sample from each plot was collected 3 days (1996), 9 months (1997), 6 years (2002) and 12 years (2008) after the fires. In 2009, soil samples from four cultivated fields close to the old fields were also taken to represent the initial conditions before the abandonment. At all times, we took soil samples in a limited area (about 2 × 2 m) inside each plot with a volumetric prismatic soil auger (5 × 5 × 30 cm). The cores (0–30 cm depth) were divided into four layers: 0–2.5; 2.5–5; 5–15 and 15–30 cm for soil samples

Table 1 Annual rainfall (mm), mean annual temperature (°C), annual potential evapotranspiration (mm) and annual water deficit (mm) for 1996–2008. Meteorological station: Os de Balaguer-Monestir d'Avellanès (41° 52' N, 0° 45' E, 580 m a.s.l.)

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Rainfall (mm)	795.4	633.6	386.8	517.2	532.4	500.6	561.7	710.6	376.6	366.4	353	350.4	634.4
Temp (°C)	12.7	13.8	13.0	12.9	13.2	13.0	13.1	13.6	12.7	12.3	13.4	13.0	18.7
ETP (mm)	641.2	675.0	652.1	650.2	660.5	658.2	651.7	680.6	643.4	638.1	674.1	651.5	1046.5
Water deficit (mm)	169.4	240.8	329.4	217.1	299.2	301.4	131.0	290.5	384.9	350.4	356.7	370.8	524.9

from 2002 and 2008. In 1996 and 1997, soils were only sampled to a depth of 5 cm.

Soil measurements

Soil samples were air-dried and sieved (2 mm) before analyses. Bulk density and stoniness were only measured in 2002 and 2008 by a volumetric auger of 5×5 cm section. As we did not measure the bulk density and stoniness in all samples we obtained the following regression (4) from soils of 2002 and 2008 and we used it to calculate bulk density of the fine earth Bdf_e in g fine earth cm⁻³ fine earth:

$$Bdf_e = 0.6180 (0.2167) + 0.7517 (0.1782) \exp^{-0.3124 (0.1748) SOC_{conc}} \quad (4)$$

$$p < 0.0001 \\ R^2 = 0.1515$$

where SOC_{conc} is the soil organic C concentration (%).

The stone content of each soil was calculated from the mean of 2002 and 2008 samples and assumed constant per soil layer and vegetation type. The percentage of stoniness was transformed to the volume of stones by Eq. 5 (Cabidoche 1979):

$$V_s = \frac{S}{D_s} \frac{100}{D_s \frac{S}{D_s} + \frac{100-S}{Bdf_e}} \quad (5)$$

where Bdf_e is the bulk density of the fine earth (g fine earth cm⁻³ fine earth), V_s is the stone volume (%), S is the Stoniness (%) and D_s is the particle density (2.65 g cm⁻³). Then, soil organic C content (Mg C ha⁻¹) was calculated by Eqs. 6 and 7:

$$SOC_{cont} = \frac{SOC_{conc} V_{fe} Bdf_e d}{100} \quad (6)$$

$$V_{fe} = 100 - V_s \quad (7)$$

where SOC_{cont} is soil organic C content (Mg C ha⁻¹), V_{fe} the volume of fine earth (%) and *d* is the soil depth (cm).

A subsample of the fine earth of each sample was finely ground in an agatha mortar to analyze total organic C by dichromate oxidation (Moebius 1960)

adapted to a colorimetric method (Soon and Abboud 1991). To reduce the analytical variability among sampling periods, all samples from 1996, 1997 and 2002 were recovered from storage and analyzed in the same batch as the samples from 2008. Soil samples from 1997 were only analyzed for the burned plots, as the soils from the unburned plots were not available.

Modeling

We selected the RothC-26.3 model (Coleman and Jenkinson 1996) to simulate the changes over time of soil organic matter in the abandoned fields. The model was used as a tool to interpret the mechanisms that induce the changes in soil organic C that we observed in the first 5 cm of soil. The RothC model uses monthly input data and is sensitive to climate, plant productivity, plant cover, plant litter quality and soil clay content. For the meteorological data, we used a monthly series from the meteorological station in *Monestir de les Avellanes*.

To run the RothC model, we calculated the pool of inert organic matter (IOM) for each soil using the equation of Falloon et al. (1998). Then, we ran the model to equilibrium with the average climate data for the study area, and we fitted it to the measured values of C in cultivated fields. We assumed a steady state at the time of abandonment (1960) for all sites. Then, we simulated the accretion process that occurred once the agricultural practices had ceased. We considered that the implantation of new vegetation had different rates depending on the plant community. Grasslands would be established during the first year after abandonment and mixed shrub-grasslands and shrublands would take from 4 to 6 years to develop. During this period, shrublands would go through a grassy period with plant residue inputs equivalent to grasslands. They would gradually incorporate the inputs of the respective shrubs. To decide which plant quality factors (DPM/RPM ratio) to use, we considered the N content of each type of vegetation. Then, plant residue inputs per plant community during aggradation were obtained by fitting the model to the three sample dates (1996, 2002 and 2008) for the grassland community. For the other two communities, the model was fitted to the two first sample dates (1996 and 2002) while the increase observed between 2002 and 2008 was fitted separately, as explained below. To simulate the drought period from 2004 to

2008, we applied a correction factor to the organic matter inputs, as we considered that drought caused a decrease in shrub biomass. Thus, in each of the drought years, an increasing proportion the standing shrub biomass would be incorporated into the soil as plant derived inputs.

To simulate the effect of fires on the burned plots, we introduced organic matter inputs of C due to fire into the model. The organic C inputs were calculated by the difference in soil organic C between burned and unburned plots just after the fires since we considered that this was the part of the organic carbon that was incorporated to the soil after the fires. To account for the lower decomposability of the organic matter pool deposited after fire we slightly reduced the DPM/RPM ratio just after the fire. We observed that after the fires, plant recolonisation of resprouting grassland and mixed shrub-grassland were faster than that of seeder shrubland (Casals 2002). The list of parameters that were used for the simulations can be found in Tables A, B and C in Online Resources 1.

Above-ground plant biomass was measured in 2009 in each plant community. Grassland biomass (mainly *B. retusum*) was measured by using four squares of 33×33 cm within each plot and clipping all the above-ground biomass. All clipped material was oven-dried at 60°C for 48 h and weighed. For the shrubs, above-ground biomass (*G. scorpius* from mixed shrub-grassland plots and *R. officinalis* from shrubland plots), we cut 40 individuals of *R. officinalis* and 40 of *G. scorpius* and we measured the basal diameter and the dry weight (60°C for 48 h). With these values, we calculated the allometries for the total and the green biomass of both species (see Table D in Online Resources 1). Then, to calculate the biomass, we measured the basal diameters of all shrubs located inside a square of 1×1 m in each of the burned plots.

Statistical analysis

We used general linear model (GLM)-repeated measures analysis to test the effects of time, fire and plant community on soil organic C stocks. A GLM-repeated measures analysis was also used to test the effects of time and soil depth in each plant community. Time was used as a within-factor and fire, plant community and soil depth were considered between-factors in their respective analyses. Our data was normally distributed. Prior to analyses, all data was

tested for homogeneity of variances by using Levene's test. Original data was log-normal transformed when variances were unequal. The data did not fail the sphericity test, so the assumed sphericity option was used. When significant, multiple comparisons were performed using the Bonferroni method. The probability threshold used to determine significance was $p < 0.05$.

The fit between Roth-C simulations and measurements was tested by the mean difference test (root mean square error, RMSE, Smith et al. 1996). We also tested the consistent over- or under-prediction errors of the model (bias), by calculating the mean difference (MD) between measurements and simulations. The significance of this difference was evaluated by the Student's *t*-test (Smith et al. 1996). All statistical analyses were performed with PASW Statistics 17.

Results

Temporal changes of soil organic C

For the first 36 years after the abandonment of agricultural practices, and assuming initial stocks similar to those of actual neighbour agricultural fields, there was an increase in soil carbon stocks (0–5 cm) in all plant communities (Table 2 and Fig. 2). C sequestration rates ranged from 0.113 to 0.153 Mg C ha⁻¹ yr⁻¹. In 1996, before experimental fires, there were no differences in organic C stocks in the unburned soils among plant communities and no changes were observed in any of the plant communities in the following 6 years (Table 2). In contrast, in 2008, soil organic C increased in both mixed shrub-grasslands and shrublands, while no changes were observed in pure grasslands.

The observed C sequestration rates between 1996 and 2002 for all the unburned communities and between 2002 and 2008 for the pure grassland were not calculated because the differences between observations were not significant. Simulated sequestration rates after 1996, which used the same plant material inputs, were much lower than those that occurred during the first 36 years after abandonment. However, for the period between 2002 and 2008, observed sequestration rates and those simulated with increased C inputs showed much higher values in mixed-shrub grasslands and shrublands (Table 2). The RothC

Table 2 Soil organic C content (SOC cont) in the first 5 cm of soil in 1996, 2002 and 2008 and C sequestration rate in different periods (1960–1996; 1996–2002 and 2002–2008) calculated with the observed values and the simulated values

(using constant C inputs or increasing C inputs over time) in unburned and burned plots for each plant community. C content of soil in 1960 derived from measurements of cultivated fields in 2009. Mean and S.E. in brackets ($n=5-6$)

Plant community	SOC cont. (Mg C ha ⁻¹)			C sequestration rate (Mg C ha ⁻¹ yr ⁻¹)					
	1996	2002	2008	1960–1996 Observed	1996–2002		2002–2008		
					Observed	Simulated	Observed	Sim. increased inputs	Sim. constant inputs
Unburned									
Grassland	8.99 (0.61)	8.42 (0.92)	8.88 (0.91)	0.148	n.s.	0.053	n.s.	–	0.050
Mixed shrub-grassland	7.73 (0.90)	8.89 (0.71)	13.74 (1.52)	0.113	n.s.	0.057	0.808	0.923	0.053
Shrubland	9.20 (0.60)	7.16 (0.93)	12.48 (1.32)	0.153	n.s.	0.043	0.886	0.557	0.042
Burned									
Grassland	10.11 (0.41)	8.01 (0.61)	10.26 (0.70)	0.179	–0.351	0.023	0.376	0.005	–
Mixed shrub-grassland	10.98 (0.51)	7.73 (0.60)	11.27 (0.71)	0.203	–0.541	–0.133	0.590	0.205	–
Shrubland	11.41 (1.00)	8.18 (0.67)	11.87 (0.60)	0.215	–0.540	–0.208	0.616	0.362	–

n.s. referred to non significant

model was able to simulate this increase only after including increased C inputs (Table 2).

The effects of fire were only significant in 1996, just after the fires (Fig. 1), and consisted of organic C increases in the burned plots. These increases were greater in soils from mixed shrub-grasslands and shrublands than in soils from the pure grasslands. Although we were not able to reanalyse unburned soils from 1997, no differences were found at that time between burned and unburned plots in the first 2.5 cm of soil (Casals 2002).

Burned plots showed decreases in organic C between 1996 and 2002 in all plant communities (Fig. 2). Similarly to the unburned plots, between 2002 and 2008 there was a significant soil organic C increase in the first 5 cm, which in this case affected all vegetation types—including pure grasslands—but of lower magnitude than in unburned soils (Table 2).

Soil organic C in the first 30 cm

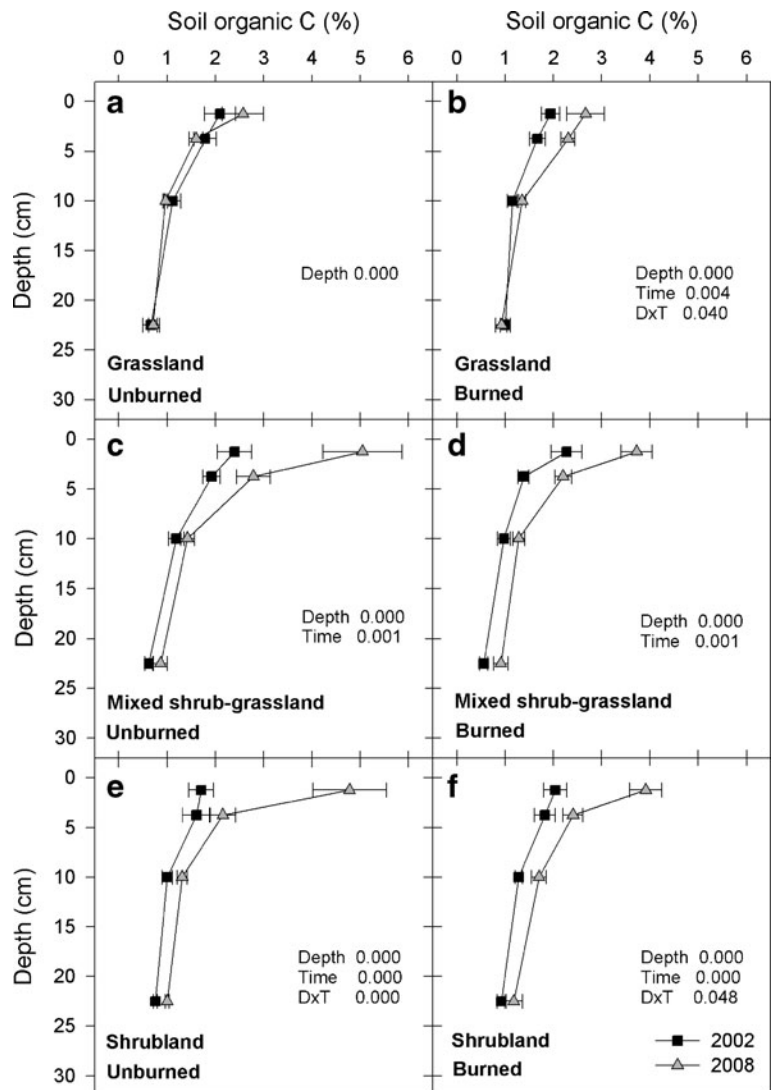
In all the soils, organic C was higher at the soil surface than in subsurface layers (Fig. 1). We did not find significant differences between burned and unburned plots in 2002 or in 2008. However, when we compared the results from 2002 and 2008, we observed that the surface accumulation of organic

matter in 2008 was greater than in 2002, mainly in the plant communities with shrubs (Fig. 1 C–F). Although the main differences in organic C were in the first layers, the mixed shrub-grasslands and the shrublands showed significant differences, even in deeper layers. The highest organic C accumulation in the soil surface occurred in 2008 in the plant communities with shrubs (shrublands and mixed shrub-grasslands), in which organic C reached values of up to 5% in the first 2.5 cm of soil (Fig. 1 C,E).

Simulation of soil organic C using the RothC model

Assuming steady state, we obtained a mean annual input of necromass for the crops of about 0.22 Mg C ha⁻¹ yr⁻¹ (Table 3). This value included cereal residues and roots. The Roth C model was able to simulate the measured C increases due to the abandonment of agriculture by using annual inputs of organic C that ranged from 0.68 to 0.84 Mg C ha⁻¹ yr⁻¹, depending on the community (Table 3). These values were in all cases much lower than the standing above-ground biomass. In the burned plots, from 1996 onwards, the model was able to predict the increases observed in all vegetation types, as well as the first year decline observed in mixed-shrub grasslands and in shrublands (Fig. 2). When we ran the model with constant C input

Fig. 1 Soil organic C concentration (%) at different depths in 2002 and 2008 (6 and 12 years after the fires) and for each plant community (grassland, mixed shrub-grassland and shrubland). Significant factors and interactions in GLM-Repeated Measures are indicated. Mean \pm SE ($n=5-6$)



values, soil C stocks showed a slight increasing trend in all unburned plant communities for the period from 2002 to 2008 (Table 2). However, we only got a reasonable fit to the observed values in the pure grassland community (Fig. 2).

To fit the simulations to the high values of soil organic C that were observed in 2008 in the woody plant communities (shrubland and mixed shrub-grassland), we increased the organic C inputs in the simulations for both unburned and burned plots. The increases in organic C inputs were gradually introduced into the model, as we assumed that the effects of drought increasingly affected the standing biomass of shrubs. Thus, we ran the model with a linear and cumulative organic C input increase.

After fitting the model to the observed C stocks, we observed that the simulated inputs for 2008 were much lower than the observed sequestration values, except in unburned mixed shrub-grasslands in which the model slightly overestimated the observed values (Table 2).

Discussion

Soil organic C content

The plant communities that we studied were the result of a secondary ecological succession process after the abandonment of the cultivated fields. The experiment

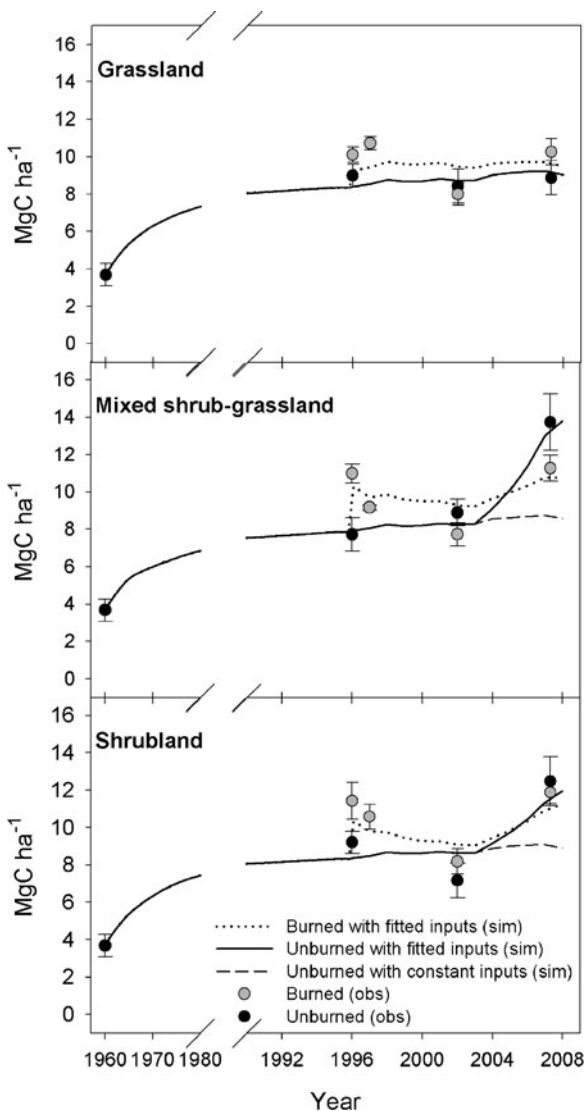


Fig. 2 Fits of the RothC model simulations to the measured soil organic C content (Mg C ha^{-1}) in the uppermost 5 cm of soil. Black circles (unburned plots) and grey circles (burned plots) show the measured organic C (Mg C ha^{-1}). Dashed lines show the fits of the RothC simulations in the unburned treatment with no variation in the C inputs over time. Solid lines (unburned sites) and dotted lines (burned sites) show the fits of the RothC simulations with increasing C inputs from 2004 to 2008. Mean \pm SE ($n=5-6$)

began in 1996, 36 years after the abandonment of agriculture. Various plant communities coexisted in the same study area at that time. About 30 years after abandonment, the organic C content in our soils ranged from 1.7% in grasslands and 2.2% in shrublands. Duguay et al. (2007) observed similar C content values in early abandoned areas dominated by similar shrubland

ecosystems. However, these values were much lower than those in forest ecosystems in the same climatic area, which showed an average of 5.6% of organic C in the first 20 cm of soil (see Romanyà et al. 2007).

Fire effects

The intensity of our experimental fires ranged from low to moderate (Romanyà et al. 2001). The differences in soil organic C between burned and unburned plots were only detected in the very short term after the fire (Fig. 2), and were due to depositions of partially burned plant material. Other authors found increases in soil organic matter after moderate intensity fires (Almendros et al. 1988, 1990; Knicker et al. 2005), which were as high as a 30% increase in crown fires (Chandler et al. 1983). In contrast to other authors who found that soil organic C remain higher in burned areas for 10 years after fire (Johnson and Curtis 2001), we only detected differences between burned and unburned plots during the first year after the fire. Our results suggest that post-fire increased soil organic C may have decayed quickly, as there were no significant differences between burned and unburned plots from 2002 (6 years after the fire) onwards. This suggests that the post-fire deposition of C in our fires would have been moderate and quite labile as it decomposed quickly after the fire. As burned and unburned plots practically followed the same trend over time, we suggest that, after the first year, fires did not cause any major change in the balance between plant production and decomposition. Other studies about fire in Mediterranean shrublands did not find any differences in soil organic C in the midterm (9 years after fire) (Duguay et al. 2007).

Drought effect

Litter fall has often been related with climate (Bray and Gorham 1964; Kouki and Hokkanen 1992; Meentemeyer et al. 1982; Pausas 1997). Hence, it has widely been accepted that drought periods promote higher litter fall than wet periods. On the other hand, drought periods have been shown to decrease microbial biomass (Bottner 1985), enzyme activities (Zornoza et al. 2006) and can modify functional diversity of soil microbes (Zak et al. 1994). Indeed, some studies have described that drought periods can cause decreases in microbial

Table 3 Organic C content in the above-ground biomass in unburned plots in 1996 and C in the above-ground biomass and green biomass in burned plots in 2009 and fitted C inputs used in the simulations for each plant community

	Unburned plots (1996)	Burned plots (2009)		Fitted C inputs prior to fire (Mg C ha ⁻¹ year ⁻¹)
	C in above-ground biomass (Mg C ha ⁻¹)	C in above-ground biomass (Mg C ha ⁻¹)	C in green biomass (Mg C ha ⁻¹)	
Crops	–	–	–	0.22
Grassland	1.08	–	–	0.80
Mixed shrub-grassland	6.34	1.56	0.69	0.84
Shrubland	8.87	6.13	2.25	0.68

activity due to the low soil moisture (Sardans et al. 2006). These decreases in microbial activity can in turn reduce soil organic matter decomposition. The water deficit values that we calculated in our study area showed a major drought period from 2004 to 2008 (Table 1) that occurred after a wet period ending in 2003. By the end of the drought period we observed that a relevant proportion of the shrubs were completely dried. This increase in shrub senescence coincided with large increases in soil organic C in the first 5 cm of depth mainly in plant communities with shrubs. As these increases occurred mainly in soils of shrublands with high aboveground biomass we suggest that they may be mainly driven by the high transient input of organic matter coming from senescent shrubs. Decreases in microbial biomass associated to drought may have also favoured soil organic matter increases, however according to the Roth-C simulations the changes in microbial biomass associated to climate are of much lower magnitude than changes associated to the increased inputs. Although during drought soil microbial activity can be reduced, it may quickly increase to extremely high values as a result of rewetting the soil (Casals et al. 2000). Low microbial activity occurring during most of the drought period may be counteracted by increased microbial activity during rewetting episodes.

Root systems are usually well-developed in Mediterranean plants, in which below-ground biomass can represent more than 50% of the total biomass in grasslands dominated by *Brachypodium retusum* (Caturla et al. 2000) or in shrublands dominated by *Rosmarinus officinalis* (Sánchez-Blanco et al. 2004). Thus, the incorporation of root dead biomass in

shrublands could account for the increases in soil C as a result of drought. This pool of C may produce transient large increases in soil C at the soil surface layer, and it may also be a relevant source of C in subsurface layers. The low magnitude increases in soil organic C, that have been observed at a depth of 30 cm especially in the shrublands, suggests differential root patterns between grasslands and shrublands. Indeed, it has been claimed that shrublands show deeper rooting patterns than grasslands (Jackson et al. 1996).

The C sequestration rate during drought in the shrublands was higher in unburned than in burned plots (see Table 2), which suggests that the effects of drought were greater in older plants than in plants that regenerated after the fires (from 7 to 11 years old). This lower sensitivity to drought may be due to lower above-ground biomass or higher physiological resistance to water stress. A recent study with the Mediterranean shrub (*Cistus clusii* Dunal), indicates that drought periods cause higher oxidative stress in old plants than in younger ones (Munné-Bosch and Lalueza 2007).

The C sequestration rate during drought in unburned shrublands was very high (Table 2) and amounted to 12.7% and 9.9% of the 1996 standing above-ground biomass for the mixed shrub-grassland and shrubland respectively. During the four year drought, this would represent a 50.8 % of the standing biomass for the pure shrublands and a 39.6% for the mixed shrub grassland. On the other hand, the burned plots biomass in 2009 showed a much higher recovery for the pure shrubland than for the mixed shrub-grassland (Table 3). These differences in recovery may be due to different regrowth rates and to the

effects of drought itself on plant biomass survival. Post-fire regrowth of the resprouter *G. scorpius* is expected to be faster than that of the seeder *R. officinalis*. Besides, the fitted C inputs post-fire (necromass production) were much higher in the mixed-shrubland than in the pure shrubland (Tables B and C in Online Resources 1). Thus, the reduced biomass in the mixed shrub-grasslands suggests that drought may have had a stronger effect on *Genista scorpius* than on *Rosmarinus officinalis*. Indeed, unlike the resprouter *Genista scorpius*, the geographic distribution of the seeder *Rosmarinus officinalis* includes semiarid regions. A study in young shrubs from the Californian chaparral showed high mortality (up to 80% of standing biomass) during the driest months (from July to October), when the plants were grown with high insolation (Pratt et al. 2008). This study also showed greater mortality of facultative resprouter species than seeder species, as resprouters have lower tolerance to water stress. Another study that compared 33 Mediterranean woody species (Paula and Pausas 2006) also found higher potential for structural resistance to drought and higher water-use efficiency in non-resprouters than in resprouters. Among other Mediterranean seeder species, *Rosmarinus officinalis* has a mixed or dual root system that seems to confer some advantages in the seasonal Mediterranean droughts (Guerrero-Campo et al. 2006; Palacio et al. 2007).

In shrubland plant communities, the increase in organic C pools associated with drought showed a much higher rate of C sequestration than the rate observed during the first decades after the cease of agricultural practices (Table 2). C sequestration rate was also higher than the increases in soil organic C observed soon after the fire (see Fig. 2). The increases in soil C occurring after the fire did not last for more than six years, as burned soils showed similar values to unburned soils after this time. In contrast, we do not know whether the large pools of organic C entering the soil as a result of drought will remain in the soil for a long time or will quickly decompose. The rate of plant regrowth after drought and the soil organic C stabilization capacity associated with clay and carbonates may be relevant issues to consider in the study of C pool balances in the long term in Mediterranean shrublands affected by intense drought periods. As the effects of drought will not enhance NPP in any case, we expect that the consequent

increased inputs of C in soil will be transient and thus will not be sustained under a prolonged drought.

Conclusions

Forest fires in Mediterranean grasslands and shrublands have caused short-term increases in the soil organic C resulting from partially burned plant material. These changes quickly decreased after the fire.

In plant communities with shrubs, climatic variability has greatly influenced soil organic C. In these systems, drought periods can transiently increase organic C inputs in soils. The magnitude of the drought effects will primarily depend on the maturity of the shrubland and secondarily on the drought sensitivity of each shrub species. Organic C in Mediterranean pure grasslands was less sensitive to drought.

Soil organic C stocks in woody plant communities were more sensitive to climatic variability than to a single fire event. In the frame of global warming, more studies need to be done to find out the long-term relevance of the pulses of C entering the soil that are associated with drought periods.

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Supplementary material

Table A. List of parameters used in the grassland community to run the RothC-26.3 model from 1960 to 2008. Meteorological data are not included in the table.

	Until 1960	1961-1996	1996 (fire)	1997	1998	1999	2000	2001	2002-2008
<i>Unburned plots</i>									
Plant community	cultivated fields	grassland	grassland	grassland	grassland	grassland	grassland	grassland	grassland
Clay content (%)	27.1	18.6	18.6	18.6	18.6	18.6	18.6	18.6	18.6
DPM/RPM ratio ¹	1.44	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
<i>Burned plots</i>									
Plant community	cultivated fields	grassland	grassland/fire	early grassland	early grassland	early grassland	early grassland	early grassland	grassland
Clay content (%)	27.1	18.6	18.6	18.6	18.6	18.6	18.6	18.6	18.6
DPM/RPM ratio ¹	1.44	0.67	0.67	0.50	0.67	0.67	0.67	0.67	0.67
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated except Oct.	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	1.89	1.08	1.03	0.97	0.91	0.86	0.80

¹DPM: Decomposable plant material

RPM: Resistant plant material

DPM/RPM: Decomposability of incoming material

²Plant residues inputs are expressed as TmC ha⁻¹ year⁻¹

Table B. List of parameters used in the mixed shrub-grassland (mixed s-g) community to run the RothC-26.3 model from 1960 to 2008. Meteorological data are not included in the table.

	Until 1960	1961-1964	1965-1995	1996 (fire)	1997	1998	1999
<i>Unburned plots with constant inputs</i>							
Plant community	cultivated fields	grassland	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g
Clay content (%)	27.1	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.44	0.67	1.40	1.40	1.40	1.40	1.40
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.84	0.84	0.84	0.84	0.84
<i>Unburned plots with fitted inputs</i>							
Plant community	cultivated fields	grassland	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g
Clay content (%)	27.1	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.44	0.67	1.40	1.40	1.40	1.40	1.40
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.84	0.84	0.84	0.84	0.84
<i>Burned plots with fitted inputs</i>							
Plant community	cultivated fields	grassland	mixed s-g	mixed s-g/fire	early mixed s-g	early mixed s-g	early mixed s-g
Clay content (%)	27.1	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.44	0.67	1.40	1.40	1.00	1.40	1.40
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	vegetated except Oct.	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.84	4.05	0.96	0.94	0.91

	2000	2001	2002-2003	2004	2005	2006	2007	2008
<i>Unburned plots with constant inputs</i>								
Plant community	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g
Clay content (%)	20.3	20.3	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.40	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Soil cover	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5
Plant residues inputs ²	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
<i>Unburned plots with fitted inputs</i>								
Plant community	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g
Clay content (%)	20.3	20.3	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.40	1.40	1.40	0.67	0.67	0.67	0.67	1.40
Soil cover	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5
Plant residues inputs ²	0.84	0.84	0.84	1.47	2.11	2.74	3.38	4.02
<i>Burned plots with fitted inputs</i>								
Plant community	early mixed s-g	early mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g	mixed s-g
Clay content (%)	20.3	20.3	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.40	1.40	1.40	0.67	0.67	0.67	0.67	1.40
Soil cover	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5
Plant residue inputs ²	0.89	0.86	0.84	0.99	1.14	1.30	1.46	1.62

¹DPM: Decomposable plant material

RPM: Resistant plant material

DPM/RPM: Decomposability of incoming material

²Plant residues inputs are expressed as TmC ha⁻¹ year⁻¹

Table C. List of parameters used in the shrubland community to run the RothC-26.3 model from 1960 to 2008. Meteorological data are not included in the table.

	Until 1960	1961-1968	1969-1995	1996 (fire)	1997	1998	1999
<i>Unburned plots with constant inputs</i>							
Plant community	cultivated fields	grassland	shrubland	shrubland	shrubland	shrubland	shrubland
Clay content (%)	27.1	21.3	21.3	21.3	21.3	21.3	21.3
DPM/RPM ¹	1.44	0.67	0.25	0.25	0.25	0.25	0.25
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.68	0.68	0.68	0.68	0.68
<i>Unburned plots with fitted inputs</i>							
Plant community	cultivated fields	grassland	shrubland	shrubland	shrubland	shrubland	shrubland
Clay content (%)	27.1	21.3	21.3	21.3	21.3	21.3	21.3
DPM/RPM ¹	1.44	0.67	0.25	0.25	0.25	0.25	0.25
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.68	0.68	0.68	0.68	0.68
<i>Burned plots with fitted inputs</i>							
Plant community	Cultivated fields	Grassland	shrubland	shrubland/fire	early shrubland	early shrubland	early shrubland
Clay content (%)	27.1	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	1.44	0.67	0.25	0.25	0.20	0.25	0.25
Soil cover	vegetated except July, Aug., Sept.	vegetated	vegetated	Vegetated except Nov., Dec.	vegetated except Jan., Feb.	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5
Plant residue inputs ²	0.22	0.80	0.68	2.85	0.46	0.50	0.54

	2000	2001	2002-2003	2004	2005	2006	2007	2008
<i>Unburned plots with constant inputs</i>								
Plant community	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland
Clay content (%)	21.3	21.3	21.3	21.3	21.3	21.3	21.3	21.3
DPM/RPM ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soil cover	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5
Plant residue inputs ²	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
<i>Unburned plots with fitted inputs</i>								
Plant community	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland
Clay content (%)	21.3	21.3	21.3	21.3	21.3	21.3	21.3	21.3
DPM/RPM ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soil cover	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5
Plant residues inputs ²	0.68	0.68	0.68	0.98	1.29	1.60	1.91	2.21
<i>Burned plots with fitted inputs</i>								
Plant community	early shrubland	early shrubland	shrubland	shrubland	shrubland	shrubland	shrubland	shrubland
Clay content (%)	20.3	20.3	20.3	20.3	20.3	20.3	20.3	20.3
DPM/RPM ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soil cover	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated	vegetated
Depth (cm)	5	5	5	5	5	5	5	5
Plant residues inputs ²	0.59	0.63	0.68	0.88	1.09	1.30	1.51	1.71

¹DPM: Decomposable plant material

RPM: Resistant plant material

DPM/RPM: Decomposability of incoming material

²Plant residues inputs are expressed as TmC ha⁻¹ year⁻¹

Table D. Allometric equations relating dry weight (DW) and basal diameter (BD) for the shrub plants *Rosmarinus officinalis* and *Genista scorpius*. Biomass equation: $DW=a*BD^b$. Standard errors in brackets, number of plants (N) and significance of equation ($adjR^2$ and p-value).

Species		a	b	N	adj R ²	p-value
<i>Rosmarinus officinalis</i>	Green biomass	0.4971 (0.2376)	1.6271 (0.1432)	40	0.9115	<0.05
	Total biomass	0.8572 (0.3027)	1.7842 (0.1047)	40	0.9570	<0.05
<i>Genista scorpius</i>	Green biomass	0.4427 (0.2521)	1.6975 (0.2644)	40	0.8164	<0.05
	Total biomass	0.5386 (0.1864)	2.0876 (0.1569)	40	0.9443	<0.05

Capítol 2:

Retenció a llarg termini dels reservoris de nitrogen mineral després del foc
a matollars i prats mediterranis

Chapter 2:

Long-term retention of post-fire soil mineral nitrogen pools in
Mediterranean shrubland and grassland

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Resum del capítol 2

La reserva de N mineral després del foc és rellevant pel posterior creixement vegetal. Depenent de les estratègies de regeneració vegetal, aquesta reserva pot ser immediatament utilitzada pel sistema sòl-planta o bé perduda. En aquest treball nosaltres hem estudiat la retenció en el sistema de la reserva mineral de N després del foc al llarg d'un període de 12 anys en tres comunitats vegetals mediterrànies contrastades. Així doncs, tres tipus de vegetació (prat, prat emmatat i brolla) van ser sotmesos a focs experimentals. Després es va aplicar un traçador (^{15}N) al reservori de N mineral i se'n va monitoritzar el seu destí en sòls i planta al llarg de 12 anys. La comunitat vegetal amb lleguminoses (prat emmatat) va mostrar la menor retenció de ^{15}N al sòl durant els primers 9 mesos després del foc. Entre els 6 i 12 anys després del foc una forta sequera va promoure la deposició de restes vegetals. Coincidint amb aquest període, la recuperació de ^{15}N durant en els primers 15 cm de sòl va incrementar en tots els casos excepte en els prats emmatats. Aquesta manca d'increment pot ser atribuïble a l'entrada de residus vegetals pobre en ^{15}N i a l'augment del rentat i la desnitrificació, possiblement provocat per les mates fixadores de N_2 . Després de la sequera, les capes més profundes de sòl van mostrar grans disminucions de N total i recuperació de ^{15}N al sòl, els quals van ser possiblement causats per processos de mineralització de N. Dotze anys després dels focs, les comunitats vegetals sense mates fixadores de N_2 van reciclar una significativa part del N derivat del N mineral alliberat després del foc i aquest reservori va continuar interaccionant amb el sistema sòl-planta.

Long-term retention of post-fire soil mineral nitrogen pools in Mediterranean shrubland and grassland

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Abstract

Background and Aims The post-fire mineral N pool is relevant for plant regrowth. Depending on the plant regeneration strategies, this pool can be readily used or lost from the plant–soil system. Here we studied the retention of the post-fire mineral N pool in the system over a period of 12 years in three contrasted Mediterranean plant communities.

Methods Three types of vegetation (grassland, mixed shrub-grassland and shrubland) were subjected to experimental fires. We then monitored the fate of ^{15}N -tracer applied to the mineral N pool in soils and in plants over 12 years.

Results The plant community with legumes (mixed shrub-grasslands) showed the lowest soil retention of ^{15}N -tracer during the first 9 months after fire. Between years 6 and 12 post-fire, a drought promoted plant and litter deposition. Coinciding with this period, ^{15}N -recovery in the first 15 cm of the soil increased in all cases, except in mixed shrub-grassland. This lack of increase may be attributable to the input of impoverished

^{15}N plant residues and enhanced leaching and denitrification, possibly by N_2 -fixing shrubs. After the drought, the deepest soil layer showed large decreases in total N and ^{15}N -recovery, which were possibly caused by N mineralization.

Conclusions Twelve years after the fires, plant communities without N_2 -fixing shrubs recycled a significant part of the N derived from the post-fire mineral N and this pool continued to interact in the plant–soil system.

Keywords ^{15}N -recovery · Drought · Soil organic C · Soil N · Legume · N_2 -fixing plant

Introduction

Fire is a natural or anthropogenic disturbance that causes short- and long-term changes in soil nutrient stocks and dynamics (MacKenzie and DeLuca 2006). The main changes in the N cycle in fire-affected ecosystems are detected either during or shortly after fires. Significant amounts of N are lost during fires, as N volatilization occurs at relatively low temperatures (200 °C) (Castro et al. 2006; Fisher and Binkley 2000; González-Pérez et al. 2004). Mineral nitrogen (N) increases in soil immediately after fire, mainly as ammonium (NH_4^+) released as a result of ash deposition and soil heating (Raison 1979; Rapp 1990). Vegetation mortality or damage after fire will reduce plant nutrient uptake and increase the potential for the loss of N by leaching. However, the N cycle is also vulnerable mid-term after fires since erosion,

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leaching, and N₂O emission may also contribute to the loss of this mineral N from the ecosystem (Levine et al. 1988; Neary et al. 1999).

Studies on the long-term effects of fires on soil have enhanced our understanding of N dynamics after these disturbances. Soil total N and organic C may decrease in the long-term (Duguay et al. 2007; Ojima et al. 1994), and N mineralization may also slow down in the long-term after fires, starting at 6–15 years after fires (Grady and Hart 2006) and continuing until at least 250 years after the fire (Polglase et al. 1992). While the low biochemical quality of the organic matter resulting from fire may favour long-term accrual of soil organic C and N (Rovira et al. 2012), post-fire changes in vegetation structure may slow down the long-term recovery of soil N stocks (Raison et al. 2009).

Changes in N forms occurring during or shortly after fires may have a strong influence on the long-term N cycle. Fire intensity and the onset of plant regeneration can be crucial processes for initial N dynamics. The high temperatures reached during high-intensity fires may chemically transform part of the N residues into highly recalcitrant organic N products, such as “Black Nitrogen” (Knicker 2007; Knicker 2010), which are stabilized into the most recalcitrant soil organic matter (SOM) pool (Knicker 2011). In contrast, low-intensity fires enhance the transformation of organic N forms into more available forms of this mineral N forms (Christensen 1973; Prieto-Fernández et al. 2004; Weston and Attiwill 1990), as well as increase labile and dissolved organic matter (Prieto-Fernández et al. 2004). Thus, N pools from low-intensity forest fires can be either leached to deep soil layers, recycled through microbial communities and incorporated into the soil organic N reserve, or used to build up new plant biomass.

The post-fire regeneration strategies of plants and their use of nutrients may have long-term effects on the resulting ecosystem. While seeders are nutrient-dependent and can readily use nutrients in freshly enriched ecosystems, resprouters can regrow faster after fires, as they can rely on their nutrient reserves and extensive rooting systems (Bell and Ojeda 1999; Verdaguer and Ojeda 2002). In contrast, a high abundance of legumes with a high rate of atmospheric N fixation has been reported shortly after fire in temperate ecosystems (Arianoutsou and Thanos 1996), at least in the short-term after fires (Casals et al. 2005). Thus, legumes improve soil fertility by increasing the availability

of soil N (Vandermeer 1989; Vandermeer 1990). Post-fire N₂ fixation can fully replace lost N and prevent post-fire N limitation (Binkley et al. 1982; Johnson et al. 2004). However, other studies suggest that the regrowth of N₂-fixing vegetation after fires is insufficient to compensate the N loss caused by fires, although it may partially contribute to long-term N accretion in ecosystems with low fire frequency (Johnson and Curtis 2001; Perakis et al. 2011; Wells 1971).

In this context, here we studied the fate of the post-fire mineral N pool on the plant and soil system over 12 years in three contrasted plant communities growing in abandoned fields in the Mediterranean region. The first community was dominated by resprouting grassy sward (*Brachypodium retusum*), the second combined the grassy sward with a resprouting N₂-fixing shrub (*Genista scorpius*), and the third was dominated by an obligate seeder shrub (*Rosmarinus officinalis*). We hypothesized that the fate of the N-NH₄⁺ released after fires differs depending on the plant community and the presence of N₂-fixing plants, which are able to incorporate atmospheric N into the soil N cycle. Other parameters, such as fire intensity or drought, may also affect the long-term fate of N in these plant–soil systems.

Material and methods

Study site

The study was carried out in a set of abandoned fields located in the NE Iberian Peninsula (41° 56' N, 0° 37' E, 460 m.a.s.l.). The climate is dry Mediterranean continental, with a mean annual temperature of 13.5 °C and mean annual precipitation of 516.9 mm (observation period: 1996–2008; Monestir de les Avellanes, 41° 52' N, 0° 45' E, 580 m.a.s.l.). The rainfall distribution is markedly seasonal, with maximum values occurring in spring and autumn, broken by a dry season that usually lasts from June to September. Monthly climatic data were obtained from the meteorological station mentioned previously above (10 km away from the experimental area), and the monthly water deficit was calculated using Thornthwaite and Mather's (1957) equations, assuming 100 mm of available water-holding capacity (Table 1).

The fields were on terraces that were abandoned in the early 1960s. At the beginning of the study, in 1996,

Table 1 Mean Temperature (°C), rainfall (mm) and water deficit (mm). Values are annual mean for the periods between sampling dates: first 9 months after fire, from month 9 to year

6 after fire, from year 6 to year 12 after fire; and for the 4-year drought (2004–2008). Meteorological station: Os de Balaguer-Monestir d'Avellanes (41° 52' N, 0° 45' E, 580 m a.s.l.)

	Period between samplings			Drought
	Oct. 1996–Jun. 1997 (To 9th month)	Jul. 1997–Feb. 2002 (9th month to 6th year)	Mar. 2002–Feb. 2008 (6th to 12th year)	Jan. 2004–Feb. 2008
Mean Temp. (°C)	11.1	13.1	13.1	12.6
Rainfall (mm)	566	450	451	349
Water deficit (mm)	6.5	281.0	311.4	352.5

the fields were colonized by three plant communities that are widely distributed in the Mediterranean basin, namely grassland, mixed shrub-grassland, and shrubland. The grassland was dominated by the perennial resprouting grass *Brachypodium retusum* (Pers.) Beauv. In the mixed shrub-grassland, the N₂-fixing shrub *Genista scorpius* L. in Lam et DC. was scattered over a grassy sward of *B. retusum*. Finally, the shrubland was dominated by the evergreen obligate seeder *Rosmarinus officinalis* L. The study areas had been free of fire and grazing by domestic animals for at least 10 years before the study. The soils are Calcaric Cambisol (FAO-UNESCO 1988), developed from a fine-texture Eocenic limestone and marl colluviums, with a pH (H₂O) of 8.2 and containing 60 % of calcium carbonate.

Experimental design

For each community, six pairs of plots (from 20 to 60 m²) were selected and distributed in various terraces. In October 1996, 12 experimental fires were set in the grassland and mixed shrub-grassland plots; 21 days later (November) six shrubland plots were burned after cutting all shrubs and letting them dry for a week. For each burned plot there was a paired unburned control plot, which represented vegetation and soil in an undisturbed state. During fires, temperature was measured at ground level (surface) every 30 s with four thermocouples per plot. The time-temperature curve for each type of vegetation was obtained as the mean of the four thermocouples per plot and the six plots per vegetation type (24 measurements per vegetation type). Fire intensity was low to medium, with temperatures at ground level from 300 to 526 °C. A detailed description on the initial soil N forms and fire intensity can be found in Romanya et al.

(2001). After the fires, a homogeneously burned area of 2×2 m within each burned plot was selected for isotopic labelling. In all cases this area was covered by ashes.

Soil ¹⁵N-NH₄⁺ labelling and plant and soil sampling

Given that NH₄⁺ is a direct product of combustion (Covington and Sackett 1992), we applied ¹⁵N-NH₄⁺ tracer on the soil surface of each 2×2 m subplot to label the post-fire pool of NH₄⁺ in the surface soil layer, which holds the ashes. To prevent the volatilization of ammonia, labelling was applied 3 days after the fires by adding 1 kg N ha⁻¹ of high ¹⁵N-enrichment ¹⁵NH₄Cl, 99 atom % excess. The amount of N added to the soil surface was considered negligible compared to the N changes occurring in the soil during the days after the fires. To achieve the maximum homogeneity in the labelling, we divided the subplot into 64 squares of 25×25 cm and sprinkled each area with 18.75 ml of ¹⁵NH₄Cl solution (1 l m⁻² of 0.333 mg ¹⁵N l⁻¹) (see Casals et al. (2005) for more details).

Soil samples were taken from burned plots in labelled and unlabelled subplots with a volumetric prismatic auger (5×5×30 cm) at the following times: 3 days (10 min after the labelling), 9 months, 6 years and 12 years after the fires. Soil cores were divided into four layers: 0–2.5; 2.5–5; 5–15 and 15–30 cm for the samples taken in 2002 and 2008. In 1996 and 1997, soils were sampled only to a depth of 5 cm and split into the two first layers. Bulk density was measured per each sampling date and layer by a volumetric auger with a 5×5 cm cross section.

Plant samples were collected 9 months and 12 years after the fires. We sampled only the aboveground biomass; belowground biomass was not considered. *B. retusum* aboveground biomass was sampled using

four squares of 33×33 cm and all the biomass was collected. For *G. scorpius* and *R. officinalis*, we calculated allometries with 40 individuals of each species using the basal diameter and the dry weight, and then we measured the basal diameter of all the shrubs located in a 1×1 m square. See Martí-Roura et al. (2011) for more details.

Soil and plant measurements

Soil samples were air-dried and sieved (2 mm) before analysis. Soil NH_4^+ and NO_3^- were extracted with 2 M KCl (1:5 w:v) and determined colorimetrically using a Technicon Autoanalyzer (Technicon Instruments Corp. New York, USA). A subsample of the fine earth of each sample was finely ground in an agate mortar to analyse ^{15}N -enrichment and total N. Aboveground plant samples were dried (60 °C for 48 h) and also finely ground. Soil and plant samples were then encapsulated and analysed for total N and ^{15}N -enrichment. The content (as a percentage of dry mass) and the stable isotope ratios of N were measured by an elemental analyser (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (20–20 isotope ratio mass spectrometer; PDZ Europa, Sercon Ltd., Cheshire, UK).

^{15}N -recovery calculations

To estimate the proportion of ^{15}N -tracer recovered in soils and plants, we used a calculation based on N mass, amount of ^{15}N in soil at the initial level, and ^{15}N -enrichments in soils and plants. We considered the first sampling date (10 min after the labelling) as the initial level ($t=0$). At this moment, the recovery of the ^{15}N in the soil from the theoretically applied ^{15}N was between 74 and 87 %. The loss of ^{15}N during the labelling application was not further considered. To calculate the atom % ^{15}N excess in each sampling, we used the following formula:

$$\text{atom\% } ^{15}\text{N excess} = \text{atom\% } ^{15}\text{N}_L - \text{atom\% } ^{15}\text{N}_C \quad (1)$$

where atom% $^{15}\text{N}_L$ is the concentration of ^{15}N in the labelled plots, and atom% $^{15}\text{N}_C$ is the concentration of ^{15}N in the unlabelled (control) plots.

To calculate the ^{15}N -recovery in soils, we used the following mass-balance equation, which relates the

initial ^{15}N concentration ($t=0$) to the ^{15}N concentration at each sampling date (t):

% ^{15}N recovery in soils

$$= \frac{\text{atom\% } ^{15}\text{N excess}_t * N_t}{\text{atom\% } ^{15}\text{N excess}_0 * N_0} \times 100 \quad (2)$$

N_t and N_0 are the total amount of N in soils (kg N m^{-2}) at time t and time 0. Similarly, to calculate the ^{15}N -recovery in plants, we used the following equation:

% ^{15}N -recovery in plants

$$= \frac{\text{atom\% } ^{15}\text{N excess plant} * N_{\text{plant}}}{\text{atom\% } ^{15}\text{N excess}_0 * N_0} \times 100 \quad (3)$$

where atom% ^{15}N excess plant is the concentration of ^{15}N in plants, which grew in labelled burned plots, corrected by reference samples. As reference samples we used the same plant species but grown in non-labelled burned plots. N_{plant} is the total amount of N (kg N m^{-2}) in plants and N_0 the total amount of N in soils (kg N m^{-2}) at time 0.

Statistical analysis

We used a General Linear Model (GLM)-Repeated Measures analysis to test the effects of time on N total, ^{15}N -enrichment and ^{15}N -recovery in soil and plant material for each community. Our data were normally distributed, and we used Levene's test to check homogeneity of variances before statistical analysis. When variances were unequal, original data were log-normal transformed. As the data did not fail the sphericity test, the assumed sphericity correction was used. We used the Bonferroni method to test the significance of multiple comparisons. The probability threshold used to determine significance was $p < 0.05$.

We also used a One-Way Analysis of Variance (ANOVA) to test differences in ^{15}N -enrichment, biomass and % ^{15}N -recovery between vegetation types. The same method was used to test the differences in organic C, total N and C:N ratio between burned and unburned plots. Duncan multiple range comparisons were applied for each significant factor.

Results

Changes in C and N stocks of soil

Bulk density was not altered by fire (data not shown). Three days after the fires, organic C and total N

contents in the first 5 cm of soil in grassland and shrubland showed no changes compared with unburned plots (Table 2). In contrast, soil organic C increased about 42 % in burned mixed shrub-grassland. While no changes were observed in total N, mineral N increased after fire in the mixed shrub-grassland and shrubland. This increase was mainly to changes in N-NH_4^+ , which largely increased (Table 3).

From day 3 to month 9 post-fire, organic C decreased in burned mixed shrub-grassland in the top 5 cm of soil, while no changes were found in the grassland (Fig. 1). Soil organic C in shrubland was also depleted during this period but only in the 0–2.5 cm layer (from 710 to 550 g C m^{-2} on day 3 and in month 9 post-fire, respectively). Similarly, during that time total N in mixed shrub-grassland also decreased 15 % in the top 5 cm of soil, while the other plant communities showed no changes (Fig. 1).

From month 9 to year 6 post-fire, organic C decreased slightly in the top 5 cm of soil in the grassland and shrubland, while no change was detected in total N (Fig. 1). In contrast, in the mixed shrub-grassland, organic C did not change while total N increased slightly.

From 6 to 12 years post-fire, the soil organic C content increased in the top 5 cm of soil in all three plant communities (28 % in grassland and 45 % in mixed shrub-grassland and shrubland) (Fig. 1). However, this increase occurred in both burned and unburned plots. Thus 12 years after the fires, no differences were found between burned

and unburned plots (Table 2). For all plant communities, total N in top 5 cm of soils showed a mild increasing trend from month 9 to year 12 post-fire (Fig. 1). In the mixed shrub-grassland, this trend was already significant at year 6.

In the deeper layers (5–15 cm and 15–30 cm), soil organic C increased from year 6 to 12 post-fire in mixed shrub-grassland ecosystems (from 1100 to 1300 g C m^{-2} in year 6 and 12, respectively) and in shrubland (from 800 to 1100 g C m^{-2} in year 6 and 12, respectively) (Fig. 1). In contrast, the behaviour of total N during this period differed for each layer. For all the ecosystems, total N in the 5–15 cm layer was similar for years 6 and 12. In contrast, this parameter decreased significantly from year 6 to 12 in the 15–30 cm layer (Fig. 1).

Changes in soil ^{15}N -enrichment

Overall, ^{15}N -enrichment decreased in the 0–2.5 cm soil layer of the three communities, mostly in the first year after fire. In contrast, the 2.5–5 cm layer showed an increasing trend only in the mixed shrub-grassland and shrubland (Fig. 2).

The recovery of ^{15}N -tracer in soil largely decreased, mostly during the first year (Fig. 2). Mixed shrub-grassland showed the greatest decrease, with less than half the recovery of ^{15}N compared to the other plant communities, and this low value persisted until the end of the experiment. Thus, at 9 months

Table 2 Organic C, total N and C:N ratio in the top 5 cm of soil. Different letters indicate significant differences between burned and unburned plots per time point ($p < 0.05$). Values are means (\pm SE) ($n = 5\text{--}6$)

	Time after fires			
	3 days		12 years	
	Unburned	Burned	Unburned	Burned
Grassland				
Organic C (g m^{-2})	899.6 (61.0)	1010.9 (42.5)	887.6 (91.2)	1026.4 (70.2)
Total N (g m^{-2})	98.6 (5.3)	102.1 (8.9)	119.8 (11.4)	105.6 (7.9)
C:N	9.1 (0.4)	10.2 (0.7)	7.5 a (0.4)	9.8 b (0.4)
Mixed shrub-grassland				
Organic C (g m^{-2})	772.7 a (90.0)	1098.2 b (51.4)	1373.8 (151.9)	1127.4 (70.9)
Total N (g m^{-2})	74.2 (6.1)	88.3 (4.3)	123.3 (10.9)	104.3 (5.0)
C:N	9.8 (1.1)	12.4 (0.5)	10.7 (0.5)	10.8 (0.5)
Shrubland				
Organic C (g m^{-2})	919.7 (60.5)	1141.4 (99.6)	1248.0 (131.9)	1187.4 (60.0)
Total N (g m^{-2})	74.8 (7.3)	86.0 (6.4)	111.0 (12.1)	101.3 (5.0)
C:N	12.7 a (1.1)	13.1 b (0.7)	11.1 (0.5)	11.6 (0.6)

Table 3 N-NH₄⁺, N-NO₃⁻ and N mineral in the top 5 cm of soil in unburned and burned unlabelled plots 3 days after fire. Different letters indicate significant differences between unburned and burned plots ($p < 0.05$). Values are means (\pm SE) ($n=6$)

	N-NH ₄ ⁺ ($\mu\text{g g soil}^{-1}$)		N-NO ₃ ⁻ ($\mu\text{g g soil}^{-1}$)		N mineral ($\mu\text{g g soil}^{-1}$)	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Grassland	2.79 (0.37)	3.85 (0.62)	1.16 (0.46)	2.56 (0.53)	3.95 (0.75)	6.41 (1.07)
Mixed shrub-grassland	2.44 a (0.32)	8.30 b (1.24)	1.44 (0.43)	2.59 (0.57)	3.89 a (0.60)	10.89 b (1.17)
Shrubland	0.18 a (0.04)	3.28 b (0.90)	0.36 (0.16)	0.36 (0.10)	0.55 a (0.18)	3.64 b (0.98)

post-fire, ¹⁵N-recovery in the top 2.5 cm of soil was 23 % in grassland, 9 % in mixed shrub-grassland and 17 % in shrubland. At 6 years post-fire, between 10 and 17 % of the ¹⁵N-recovery (depending on the vegetation) remained in the uppermost layer while between 22 and 34 % was recovered from the deeper layers (2.5–5; 5–15 and 15–30 cm). In the deepest layers, we observed slight decreases in ¹⁵N-recovery between years 6 and 12 post-fire in grassland (15–30 cm layer) and in mixed shrub-grassland (5–30 cm layer) (Fig. 2). For grassland and shrubland, pooling of the top three layers (0–15 cm soil) showed a significant increase in ¹⁵N-recovery from year 6 to 12 post-fire ($p=0.039$ and $p=0.027$ respectively) while for mixed shrub-grassland this parameter remained unchanged ($p=0.205$).

¹⁵N-enrichment in aboveground plant biomass

Twelve years after the fires, ¹⁵N-enrichment was still high in plants in the labelled subplots. *R. officinalis* (the main shrub of the shrubland community) showed the highest values of ¹⁵N-enrichment at 9 months and 12 years post-fire, followed closely by *B. retusum* (the main grass of grassland and mixed shrub-grassland communities) (Table 4). The N₂-fixing plant *G. scorpius* (the main shrub of mixed shrub-grassland) showed the lowest ¹⁵N values, which, at the end of the experiment, were close to natural abundance. *B. retusum* growing in the same plots as *G. scorpius* (mixed shrub-grassland communities) showed slightly lower ¹⁵N-enrichment and higher N content than *B. retusum* grown without the legume shrub.

Long-term ¹⁵N-recovery in aboveground plant biomass and soils

Twelve years after fires, large amounts of ¹⁵N-tracer, between 27 % and 63 %, were still retained in the first

30 cm of the soil profile (Table 5), whereas the recovery in aboveground plant was very low (<2 %; Table 5). Aboveground plant in shrubland showed the highest ¹⁵N-recovery (1.7 %; Table 5), while the other plant species registered negligible recovery values (<0.1 %). The poor recovery values are attributed to either low ¹⁵N-enrichment, as in *G. scorpius* in the mixed shrub-grassland, or low biomass values, as in *B. retusum* in the mixed shrub-grassland and also the pure grassland.

Discussion

Post-fire responses

As expected after fires of low to medium intensity (Almendros et al. 1988; Almendros et al. 1990; Knicker et al. 2005), the short-term changes in organic C and total N in the upper 5 cm of soil were negligible (Table 2). However, N-NH₄⁺ in the top 5 cm of soil increased by 340 % in the mixed shrub-grassland and by 1784 % in the shrubland 3 days post-fire compared with unburned plots. These increases could be explained by the deposition of ashes and the increases in soil temperature (Raison 1979; Rapp 1990). No changes were detected in the grassland. This finding could be attributed to the lower fire intensity in this community and its higher initial NH₄⁺ content. A considerable amount of this pool remained in the first 30 cm of the soil 12 years after fire, with differences related to plant community, while the amount remaining in aboveground biomass was considerably lower (Table 5).

Most of the changes in N occurred in the first months after fire, possibly because of the loss of this mineral N by volatilization or leaching (Chorover et al. 1994; Guillon and Rapp 1989; Mackensen et al. 1996; Murphy et al. 2006) or plant uptake. Between

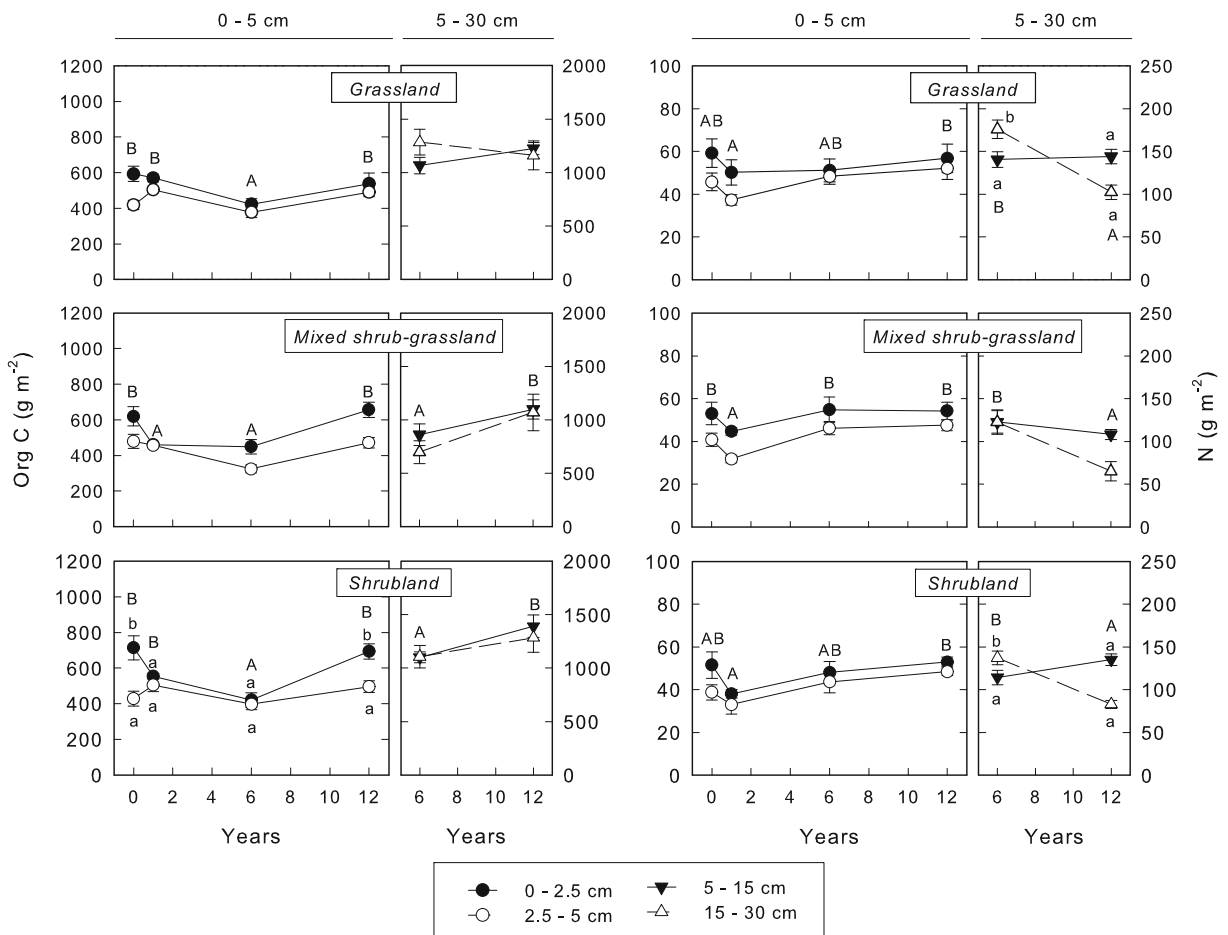


Fig. 1 Organic C and total N in soil at various depths and for each plant community (grassland, mixed shrub-grassland and shrubland) 3 days, 9 months, 6 years and 12 years after the fires. Capital letters indicate significant differences over time,

including the two layers shown in each graph, and lower case letters indicate significant differences in each layer over time. Values are means (\pm SE) ($n=5-6$)

74 and 87 % of the labelled pool of N was lost from the top 5 cm soil layer during the first 9 months. Other studies on soil N retention after ^{15}N labelling have also reported large losses (up to 40 %) during the first days of the experiment (Seely and Lajtha 1997). In our study, nitrification processes readily occurred during the first weeks after fire, followed by N-NO_3^- leaching (Romanyà et al. 2001), as heavy rains (376 mm) fell during the 3 months after labelling.

Post-fire plant regrowth can also partly account for decreases in the soil pools of ^{15}N -tracer. However, given that at 9 months post-fire the grassland and mixed shrub-grassland showed similar plant cover (Casals et al. 2005), it would appear that the highest ^{15}N -tracer losses in soils of mixed shrub-grassland are not attributable to differences in plant regrowth and

the consequent nutrient uptake. In contrast, these differences may be explained by soil processes. N-rich plant litter and burned residues of *G. scorpius* could increase N mineralization and nitrification. Increased N mineralization as a result of the addition of N-rich substrates has been widely reported (e.g. Madritch and Cardinale 2007; Kuzyakov et al. 2000). Furthermore, it has been proposed that N-enriched residues of N_2 -fixing plants promote N loss in soil by denitrification (Baggs et al. 2000; Huang et al. 2004; Millar et al. 2004; Zhong et al. 2011). Romanyà et al. (2001) did not find a short-term increase in nitrification in a mixed shrub-grassland community after fire, but this plant community showed much higher values of mineral N (Table 3) after fires than the others. Thus leaching and denitrification processes could be

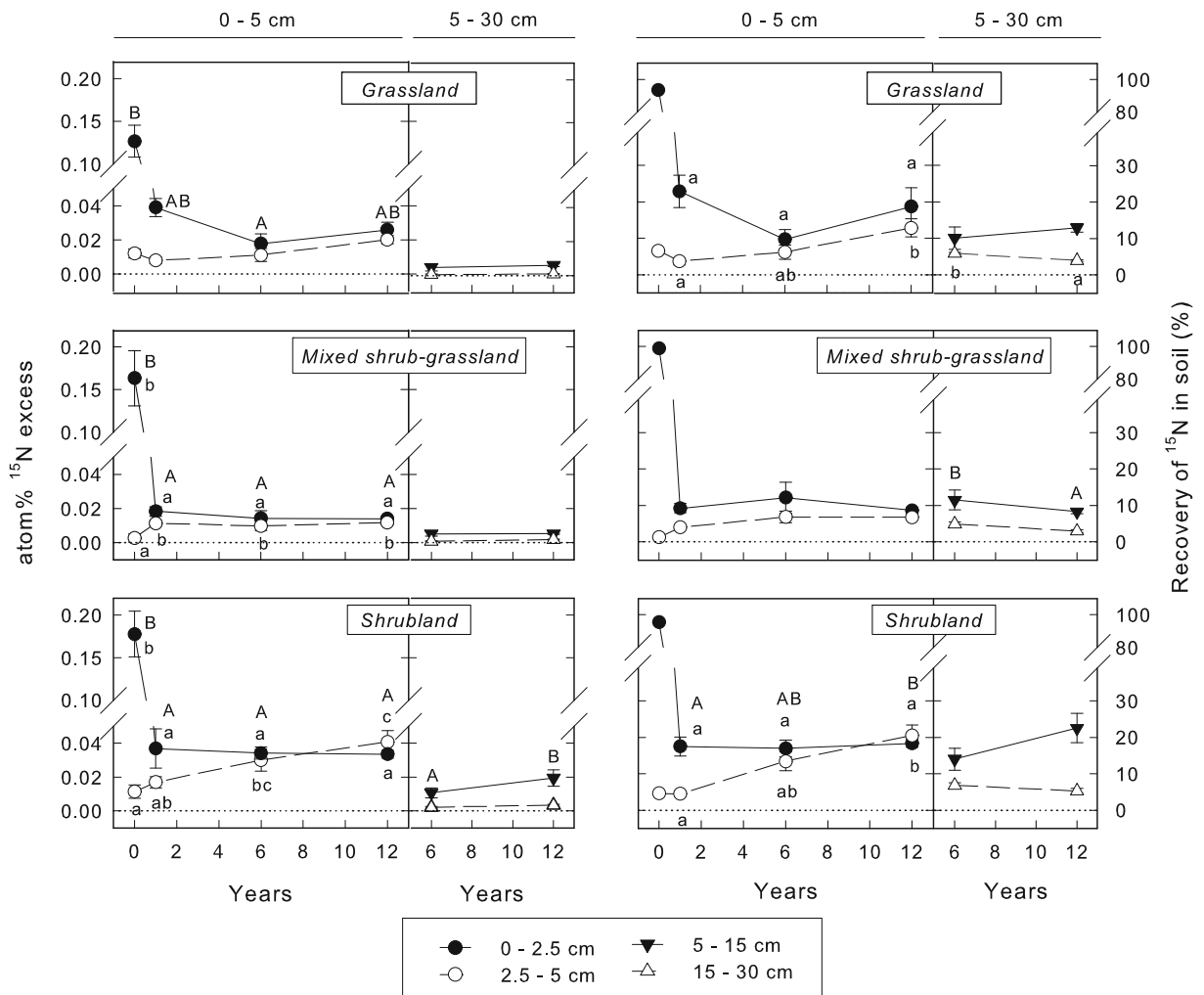


Fig. 2 ^{15}N -enrichment (Atom% ^{15}N excess) and recovery of ^{15}N (%) of the surface soil 3 days after fire, at various depths and for each plant community (grassland, mixed shrub-grassland and shrubland) 3 days, 9 months, 6 years and 12 years after

fire. Capital letters indicate significant differences over time, including the two layers shown in each graph, and lower case letters indicate significant differences in each layer over time. Values are means (\pm SE) ($n=5-6$)

involved in ^{15}N -tracer (as $^{15}\text{N-NH}_4^+$ or $^{15}\text{N-NO}_3^-$) loss, as high amounts of available mineral N were in the soil during the first months after fire when no plant uptake was possible.

Long-term changes in soil C and N pools and recycling of ^{15}N -tracer: effects of drought periods

The organic C in the top soil increased in all plant communities between years 6 and 12 post-fire; however, similar increases for total N were not observed (Fig. 1). As suggested in Martí-Roura et al. (2011), large amounts of organic matter might have been

deposited in this time as a result of a severe drought period (Table 1), which affected mainly plant communities with shrubs. These changes might be the result of an increase in belowground deposition, thus affecting the various soil layers. In the pure grassland, the increases in organic C affected only the top 5 cm of soil, where the rhizomes of this resprouting species occur. We therefore suggest that the drought-induced belowground deposition includes coarse roots in plant communities with woody plants and rhizomes of the *B. retusum* grassland. Coarse belowground plant material is generally rich in cellulose, hemicellulose or lignin and has a high C:N ratio (Birouste et al. 2012;

Table 4 Aboveground plant ^{15}N enrichment 9 months after the fires and biomass, total N and ^{15}N enrichment 12 years after the fires in plants growing in labelled plots. Values are from thedominant plants per each plant community. Within a column, values with different letter are significantly different ($p < 0.05$). Values are means (\pm SE) ($n = 5-6$)

	Time after fires			
	9 months	12 years		
	$\delta^{15}\text{N}$ (‰)	Biomass (g m ⁻²)	Total N (mg N g plant ⁻¹)	$\delta^{15}\text{N}$ (‰)
Grassland				
<i>B. retusum</i>	381.3 b (49.0)	76 a (14)	7.8 a (0.1)	34.0 b (5.0)
Mixed shrub-grassland				
<i>G. scorpius</i>	24.7 a (7.3)	334 b (53)	8.2 b (0.2)	2.8 a (0.2)
<i>B. retusum</i>	326.5 b (22.2)	121 a (50)	8.5 b (0.2)	23.1 b (4.4)
Shrubland				
<i>R. officinalis</i>	916.7 c (142.0)	1361 c (284)	6.6 a (0.4)	47.6 c (4.1)

Goebel et al. 2011; Pregitzer et al. 2002). Thus, coarse roots and rhizome deposition contribute little to the soil N pool. Moreover, new inputs of labile organic matter may promote microbial activity and mineralization of the soil N reserve. This mineralized N can be immobilized in the soil organic matter, lost by leaching or denitrification or taken up by plants. Net N mineralization will be especially high in soils with the lowest C:N ratio, as is the case of the 15–30 cm layer. In this case, increased N mineralization could account for the large decreases in N pools detected in the 15–30 cm layer in all the vegetation types. These decreases barely affected the ^{15}N -tracer pool, which showed minor or no decreases in the deepest layer. In contrast, in the top 15 cm of soil, we did not observe any effect of drought on total N but ^{15}N -recovery increased during the drought in shrubland and grassland communities (Fig. 2). This increase in ^{15}N indicates the incorporation of ^{15}N pulses from plant necromass in soil organic matter and points to immobilization processes occurring in the first 15 cm of the soil. Furthermore, these observations support our hypothesis to explain soil organic C increases during this period (Martí-Roura et al. 2011). This increase in ^{15}N -recovery occurred only in plant communities without legume shrubs. Although the inputs of ^{15}N -impoverished plant litter of the N_2 -fixing *G. scorpius* could partly explain these low values, it must be considered that the *B. retusum* sward growing in these mixed shrub-grassland community showed high $\delta^{15}\text{N}$ values, close to those of the pure grassland, and a

similar biomass and ^{15}N -recovery in plants at 12 years post-fire (Table 4). Thus, we could expect that ^{15}N -recycling in the mixed shrub-grassland through *B. retusum* litter would behave similarly to that of the pure grassland. As this was not the case, we propose that the low retention of the ^{15}N -tracer in this N_2 -fixing community is caused by the enhancement of ^{15}N loss during the decomposition of plant debris.

Legumes are an important component of post-fire successional communities (Crews 1999). These plants are able to use the increased mineral N pools caused by fire (Vitousek and Howarth 1991), but they can also provide a post-fire net N input by introducing fixed atmospheric N_2 (Hendricks and Boring 1999). The enhanced loss of N in the N -fixing plant community post-fire was negligible (less than 0.2 kg m⁻²) in terms of plant–soil N-recycling rate. Net N input

Table 5 Recovery of ^{15}N (%) in soil and plants 12 years after fire. Values are means (\pm SE) ($n = 5-6$)

	Soil	Aboveground plant		
		<i>R. officinalis</i>	<i>G. scorpius</i>	<i>B. retusum</i>
	0–30 cm			
Grassland	48.4 (7.8)	–	–	0.09 (0.01)
Mixed-Shrub-grassland	26.6 (1.5)	–	0.07 (0.01)	0.10 (0.03)
Shrubland	62.7 (7.8)	1.70 (0.32)	–	–

provided by legumes post-fire will far exceed this loss (Casals et al. 2005). Indeed, no loss of total N was observed in this plant community, and 12 years after fire the non-N-fixing plant *B. retusum* in this community had a higher N content than that found in pure grassland. This observation supports the notion that legumes enhance the regeneration of the whole community.

In conclusion, 12 years after the fires, more than half of the initial ^{15}N -tracer was still retained in the soils in communities with no legume shrubs. At that time, plants still contained part of the labelled ^{15}N - NH_4^+ released after fires, with the exception of N_2 -fixing shrubs. The presence of legume shrubs favoured loss of the ^{15}N -tracer during the first year after fire and during the drought period, which took place several years post-fire. In Mediterranean grassland and shrubland without N_2 -fixing shrubs, extreme climatic conditions, such as drought, favours the soil incorporation of plant N pools that were taken up from soils shortly after fire. The accumulation of below-ground necromass under these conditions may favour the accrual of soil organic C to a greater extent than that of N.

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Capítol 3:

Distribució i estabilització del N mineral alliberat després del foc en fraccions de mides de sòl diferents en prats i brolles mediterrànies

Chapter 3:

Post-fire mineral N allocation and stabilization into soil particle size fractions in Mediterranean grassland and shrubland

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Resum del capítol 3

A la regió mediterrània, la dinàmica del N del sòl a llarg termini després d'un incendi pot ser rellevant per l'estabilització de la matèria orgànica després del foc i pel reciclatge de N en el sistema sòl-planta. El reciclatge de N després del foc pot ser retardat per la retenció de N en fraccions de matèria orgànica física i/o químicament protegides. En el nostre cas, vam estudiar la distribució del traçador ^{15}N entre les diferents fraccions de matèria orgànica (sorra grollera, sorra fina, llim groller i llim fi i argila) i la dinàmica del traçador al llarg de 12 anys després d'unes cremes controlades realitzades en tres comunitats vegetals mediterrànies diferents. Per això, vam seleccionar 6 parcel·les per cada comunitat i vam realitzar una sèrie de cremes experimentals. Després dels focs vam aplicar $^{15}\text{NH}_4^+\text{-N}$ i vam monitoritzar el destí del traçador ^{15}N al llarg d'un període de 12 anys. A continuació, es va dur a terme un fraccionament físic en funció de la grandària de la partícula i un anàlisi de la recalitrància bioquímica del N i del ^{15}N mitjançant una hidròlisi àcida. El reservori més important de N total va ser localitzat a les partícules més fines de sòl (llim fi i argila) en ambdues parcel·les, cremades i no cremades. El foc va promoure augments del N total a les fraccions de mida mitjana mentre que les reserves de N total en fraccions més fines i més grolleres no van canviar després del foc. El traçador ^{15}N va ser ràpidament incorporat a les fraccions fines des d'on, en el cas concret de les comunitats vegetals sense lleguminoses, aquest va ser remobilitzat al llarg dels següents anys. El N recalitrant no va canviar com a conseqüència del foc, però les brolles van mostrar un marcat descens d'aquest N 6 anys després del foc. Tot i que els principals efectes a les diferents fraccions de sòl van ser detectades just després del foc, aquests van persistir fins 12 anys després dels focs. El ^{15}N novament incorporat va resultar ser menys recalitrant que el N total i, sorprenentment, les fraccions fines van tenir uns valors molt baixos de recalitrància del ^{15}N , similars als que van mostrar les fraccions grolleres. Aparentment, les transformacions del N a les fraccions més fines ($<20\ \mu\text{m}$) van se principalment regulades per la qualitat dels compostos de ^{15}N que van quedar retinguts en aquesta fracció.

Post-fire mineral N allocation and stabilisation into soil particle size fractions in Mediterranean grassland and shrubland

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Abstract

In the Mediterranean region, the long-term post-fire soil N dynamics may be relevant for soil organic matter stabilization and N cycling in the plant-soil system. The post-fire recycling of N can be hold back by the retention of N in physically and/or chemically protected fractions of soil organic matter. We studied the allocation of the post-fire ^{15}N -tracer among different soil organic matter fractions (coarse sand, fine sand, coarse silt and fine silt and clay) and the ^{15}N -tracer dynamics during 12 years after prescribed fires in three different Mediterranean plant communities. We selected 6 plots for each community and we set experimental fires. Just after the fires, we applied $^{15}\text{NH}_4^+$ -N and we monitored the fate of ^{15}N -tracer over a period of 12 years. To this end, we carried out a physical size fractionation and we analyzed the biochemical recalcitrance of N and ^{15}N by acid hydrolysis in the obtained size fraction. In both burned and unburned plots the finest soil particles ($<20\mu\text{m}$) accounted for the most of soil N. Fire promoted N increases in the medium size

fractions did not change after the fires. Interestingly, ^{15}N -tracer was quickly incorporated in fine fractions from where, in the case of plant communities free of legumes, it was remobilised in the following years. Recalcitrant N did not change due to the fire, but shrubland showed marked decreases 6 years after the fires. Despite the main effects in soil fractions were detected just after the fires, they persisted after 12 years post-fire. Newly incorporated ^{15}N -tracer was less recalcitrant than total N and, surprisingly, fine fractions had very low recalcitrant ^{15}N values, similar to the coarse fractions. Apparently, the N transformations in the finest fraction ($<20\mu\text{m}$) were mainly regulated by the quality of the ^{15}N compounds retained in the fraction.

Keywords Physical size fractionation · Soil N · ^{15}N -recovery · Biochemical recalcitrance

Introduction

Fire may cause complex alterations on physical and chemical properties of soil. Fires immediately decrease the amount of labile organic matter (either by direct combustion or thermal breakdown) and enhance both the plant N availability and the recalcitrance of organic matter pools (Almendros et al. 1992; Fernández et al. 1999). Although N volatilization is produced at relatively low temperatures (200°C) and large amounts of N can be lost during fires, mineral N pools, mainly ammonium, increase in the uppermost soil layers as a consequence of

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temperature induced mineralization and ash deposition. On the other hand, during fires soil heating can promote changes on soil N-forms to chemically more recalcitrant forms (Sánchez and Lázzari 1999) by increasing heterocyclic and aromatic N (Knicker et al. 2005). The high biochemical stability of these fire-induced recalcitrant N-forms can render them difficult to mobilize by microbial processes, and therefore able to remain in soils over years (Rovira et al. 2012). This effect may hold back primary production over the long-term (Knicker and Skjemstad 2000).

Post-fire soil mineral N, if not lost by leaching or volatilization, can be assimilated by regrowing plants or associated to soil organic matter (SOM) and stabilized. SOM stabilisation can be by physical protection or by chemical recalcitrance. Physical protection can be due to the SOM incorporation within aggregates in nanopores too small for organisms or enzymes to enter and function (Mayer 1994; Skjemstad et al. 1993) by encapsulation into hydrophobic macromolecules (Knicker and Hatcher 1997), or by the formation of organo-mineral-associates (Baldoek and Skjemstad 2000). This latter mechanism has been considered as the most important for the physical protection of organic matter (Kögel-Knabner et al. 2008). The chemical recalcitrance of the decomposition residues can also hold up SOM decomposition. Aromatic and/or O-alkyl compounds can be difficult to be metabolized by soil microbes (Almendros et al. 1984; Kalbitz et al. 2003). The prevalent view is that chemical recalcitrance plays a secondary role in SOM stabilisation (Dungait et al. 2012; Marschner et al. 2008; Mikutta et al. 2006; Rovira et al. 2010). However, in a recent field study it has been shown that N-rich labile organic matter from an incubation of labelled roots within the soil was mainly recovered in the protected fractions while the more recalcitrant fraction remained mostly unprotected (García-Pausas et al. 2012). Therefore, physical protection and chemical recalcitrance may work simultaneously and sometimes in a complex manner, because labile and recalcitrant organic pools are not evenly distributed among the physical fractions (Rovira and Vallejo 2007; Rovira et al. 2010).

Quick plant regeneration and N use post-fire can largely reduce N losses from the system. After, N will return to the soil through plant litter deposition and, depending on SOM quality, will

be retained in the soil over the short and the long-term. The interactions between plant litter and SOM pools of different nutrient richness or chemical recalcitrance can shape the microbial processes transforming the pools of soil organic matter and nutrients (Madritch and Cardinale 2007). These interactions will likely occur in diverse plant communities with contrasted litter quality such as in those with a relevant presence of legumes or combining woody and green plant material. The quality of the soil organic matter pools and of the new plant litter produced post-fire in the regrowing plant communities can regulate the post-fire allocation and stabilisation of mineral N into SOM (Zhu and Wang 2011).

We hypothesise that the stabilization of the N pool existing short-term after fire on the soil particle size fractions will depend on both the physical protection of each fraction and on the chemical recalcitrance of the SOM pools of each fraction. While N allocated to coarse fractions will be mainly stabilized by its biochemical recalcitrance, N held in the fine fractions will be mostly physically protected with little influence of its biochemical quality. Different plant communities and especially those with the presence of legumes can also influence the quality of the organic sources and SOM pools and, consequently, the N stabilization processes.

Our aim was to study the allocation and physical and chemical stabilization of the N pool existing shortly after fire among different particle size fractions over a 12-year period in three different Mediterranean plant communities (grassland, mixed shrub-grassland, shrubland). For that purpose we applied ^{15}N isotopic labelling on ashes just after fire and we assessed changes in total N and ^{15}N -tracer in different physical size fractions and in the recalcitrant pool of each fraction in the following 12 years post-fire.

Material and methods

Study site

The study was carried out at three different plant communities located in abandoned fields in the NE of Iberian Peninsula (41° 56' N, 0° 37' E, 460 m a.s.l.). The abandonment of the terraced fields was in early 1960s. The three different plant communities now occupying the old fields are representative of the Mediterranean land set

aside: grassland dominated by the perennial grass *Brachypodium retusum* (Pers.) Beauv; mixed shrub-grassland dominated by the legume shrub *Genista scorpius* L. in Lam et DC and the grass *B. retusum* and the shrubland dominated by *Rosmarinus officinalis* L. The climate is dry Mediterranean continental, the mean annual temperature is 13.5°C and the mean annual precipitation is 516.9 mm (observation period: 1996–2008; Monestir de les Avellanes, 41° 52' N, 0° 45' E, 580 m a.s.l.). The rainfall distribution is markedly seasonal, with maximum values in spring and autumn and a dry season that usually lasts from June to September. The soils are Calcaric Cambisol (FAO-UNESCO 1988), developed from a fine textured Eocene limestone and marl colluvium, with a pH (H₂O) of 8.2, 60% calcium carbonate content and the soil texture is loam. The study areas had been free of fire and grazing by domestic animals for at least 10 years before the beginning of the study.

Experimental design, soil ¹⁵NH₄⁺ labelling and soil sampling

Six pairs of plots (from 20 to 60 m²) of each vegetation type were selected in different terraces (18 pairs of plots in total). In 1996 a plot of each pair was experimentally burned while the other remained as a control plot, representing the undisturbed state. Fire intensity was low to medium, with maximum temperatures at ground level from 300 to 526°C. See Romanyà et al. (2001) for more details.

Three days after the fires, subplots of 2 x 2 m were bordered in each burned plot and a solution highly ¹⁵N-enriched (¹⁵NH₄Cl, 99 atom%, 1 kg N ha⁻¹) was applied on the ashes of these subplots to label the post-fire mineral pool of N. Subplots were divided in 64 squares (25x25 cm); each square was sprinkled with 18.75 ml of ¹⁵NH₄Cl solution (1 l m⁻² of 0.333 mg 15N l⁻¹) in order to homogenize ¹⁵N labelling on the subplots surface. See Casals et al. (2005) for more details.

From each plot, three soil cores (5x5 cm) from 0 to 5 cm depth were taken and bulked to one sample. Samplings were carried out 3 days (just after labelling), 6 years and 12 years post-fire in ¹⁵N-labelled and burned plots and 3 days post-fire in unburned plots. Then soil samples were air-dried and sieved (2 mm) before fractionation.

Particle size fractionation of soil organic matter

Size fractionation was carried out as described in Rovira and Vallejo (2002a), simplified to recover all particles <20 µm as a single fraction. Briefly, 10 g of air dried soil 2 mm sieved were softly shaken for 1 hour with 50 ml water and two glass beads. After, the soil-water mixture was placed in an ice bath and dispersed with a sonifier (100 W output, 5 minutes). The dispersed mixture was then passed through a column of meshes (200, 50 and 20 µm) under magnetic agitation and extensive water flush, thus obtaining the 2000–200, 200–50, and 50–20 µm size fractions. Each of these fractions was collected in a 20 ml flask; water was removed by siphonation with a filter coupled to a syringe in order to avoid losses of the light fraction and the whole fraction was finally dried at 60 °C. The <20 µm fraction (clay plus fine silt) was collected in 1-l jar and was recovered by flocculation with a minimum amount of a saturated solution of AlK(SO₄)₂ and later siphonation of the water phase. Finally, this fraction was recovered by centrifugation and dried at 60°C.

After particle size fractionation a subsample of each fraction was finely ground to analyze total organic C (TOC), total N and ¹⁵N enrichment. TOC was analyzed by dichromate oxidation (Moebius 1960) adapted to a colorimetric method (Soon and Abboud 1991). Total N and ¹⁵N enrichment were analyzed by an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (20–20 isotope ratio mass spectrometer; PDZ Europa, Sercon Ltd., Cheshire, UK).

Recovery of ¹⁵N calculations

To calculate total recovery of ¹⁵N in each soil fraction we considered as the initial level the first sampling date (10 minutes after the labelling). To calculate the atom % ¹⁵N excess in each sampling we used the following equation:

$$\text{atom\% } ^{15}\text{N excess} = \text{atom\% } ^{15}\text{N}_L - \text{atom\% } ^{15}\text{N}_C$$

where atom% ¹⁵N_L is the concentration of ¹⁵N-tracer in the labelled burned plots and atom% ¹⁵N_C is the concentration of ¹⁵N-tracer in the unlabelled burned (control) plots.

To calculate the percentage of ^{15}N -recovery in soils at each fraction (i) we used the following mass-balance equation that relates the initial ^{15}N -tracer concentration ($t=0$) to the ^{15}N -tracer concentration at each sampling date (t):

$$\% \text{ Recovery of } ^{15}\text{N} \text{ at the } i \text{ fraction} = \frac{\text{atom}\%^{15}\text{N excess}_t * N_t}{\text{atom}\%^{15}\text{N excess}_0 * N_0} \times 100$$

N_t is the total amount of N (g N kg^{-1}) in the soil i fraction soil at time t and N_0 is the total amount of N in soils (g N kg^{-1}) at time 0.

Biochemical recalcitrance

For analysing the biochemical recalcitrance of

SOM in each soil fraction we applied the two-step acid hydrolysis with sulphuric acid of Rovira and Vallejo (2002b). Briefly, 200 mg of dry ground soil material was refluxed with 10 ml of 2.5M H_2SO_4 for 30 min at 105°C in an aluminium heating block. The liquid was decanted after centrifugation; the unhydrolyzed residue was washed with 10 ml of water, recovered by centrifugation and dried at 60°C . This residue was gently soaked overnight with 1 ml of H_2SO_4 70-72%, at room temperature. Then, 9 ml of water were added and the residue was refluxed again, for 3 h at 105°C . After centrifugation, the liquid phase was decanted again. The unhydrolyzed residue was recovered and washed with 10 ml of water to eliminate the residual acid, the centrifugation plus decantation process was repeated. The unhydrolyzed residue

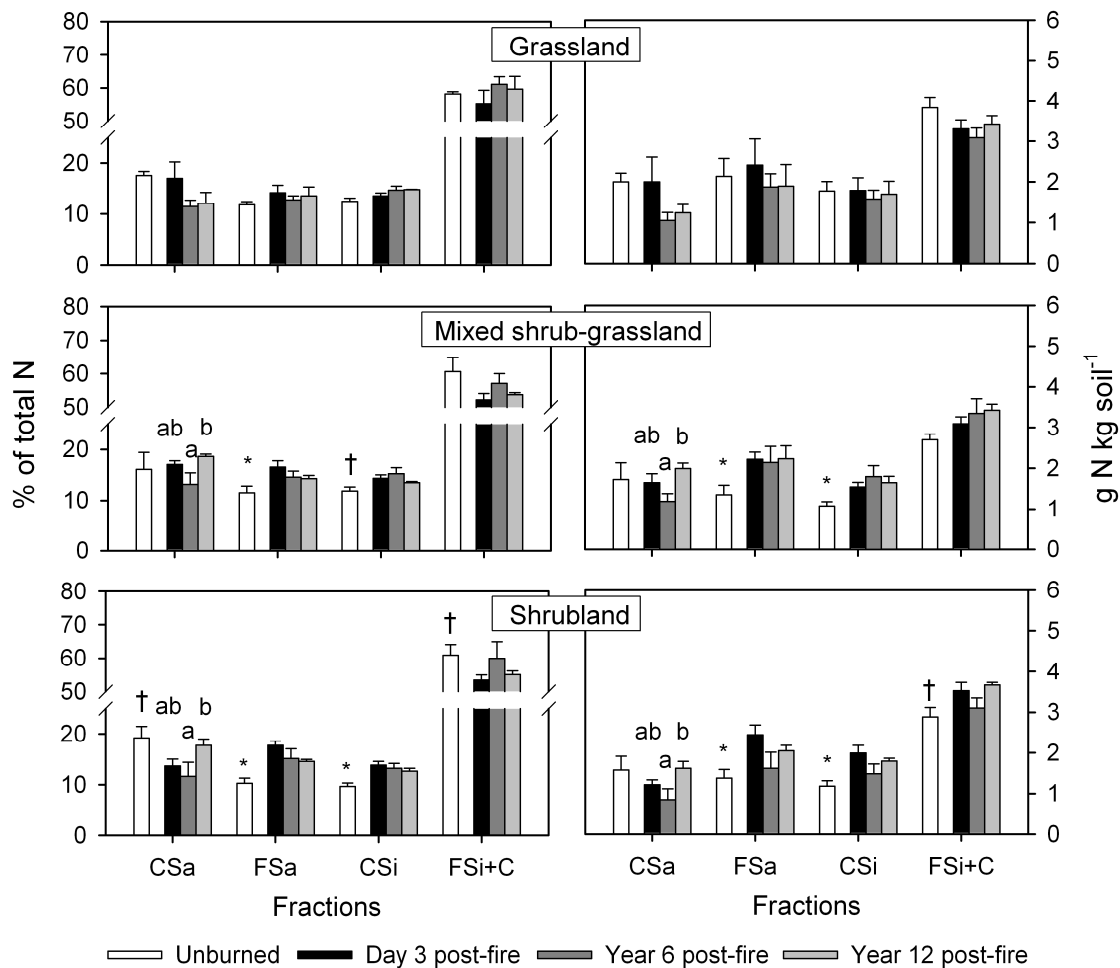


Fig. 1 Total N distribution (% of total N) in fractions (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt+clay (FSi+C)) from unburned and burned soils (day 3, year 6 and year 12 post fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with † $p < 0.1$ and * $p < 0.05$. Within a given graph, time differences are labelled with a different lowercase letter, at $p < 0.05$. Values are means \pm SE ($n=5-6$)

was finally dried at 60°C, weighed and was taken as the recalcitrant fraction. This recalcitrant fraction was analyzed for total C, N and ¹⁵N with an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (20–20 isotope ratio mass spectrometer; PDZ Europa, Sercon Ltd., Cheshire, UK). From the obtained values, the recalcitrance indices for N (RIN) and ¹⁵N-tracer (RI¹⁵N) were calculated for each fraction:

$$\text{RIN (\%)} = (\text{Unhydrolyzed N/Total N}) \times 100$$

$$\text{RI}^{15}\text{N (\%)} = (\text{Recovery of unhydrolyzed } ^{15}\text{N} / \text{Recovery of total } ^{15}\text{N}) \times 100$$

Statistical analysis

All statistical analyses were run by using PASW statistics 17. All data were checked for normal distribution (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test) before statistical analysis and, when any of both assumptions were rejected, Box-cox transformation was applied. Analysis of variance (ANOVA) was performed to test the differences

Table 1. Values of RIN (Recalcitrant Index for N) in fractions from unburned and burned soils (day 3, year 6 and year 12 post fire) and RI¹⁵N (Recalcitrant Index for ¹⁵N) from burned soils in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with † *p*<0.1 and * *p*<0.05. Time differences are labelled with a different lowercase letter, at *p*<0.05. Values are means ± SE (n=5-6)

	RIN (%)				RI ¹⁵ N (%)		
	Unburned	Day 3	Year 6	Year 12	Day 3	Year 6	Year 12
Grassland							
CSa	37.97 (2.22)	32.16 (2.31)	28.49 (0.92)	28.54 (1.39)	26.97 (2.46)	20.67 (2.30)	25.53 (1.08)
FSa	38.83 (2.04)	34.33 (1.93)	35.35 (1.33)	32.97 (1.52)	30.88 (3.06)	25.69 (2.48)	29.14 (1.51)
CSi	† 42.50 (1.49)	38.50 (1.28)	40.85 (1.12)	39.27 (0.95)	35.57 (3.24)	27.17 (2.62)	32.62 (0.78)
FSi+C	41.89 (2.00)	40.87 (1.46)	42.39 (2.73)	40.53 (2.69)	26.02 (1.20)	26.91 (1.20)	29.39 (1.59)
Mixed shrub-grassland							
CSa	† 39.53 (2.06)	33.25 (1.70)	30.77 (1.66)	30.72 (1.02)	32.93 (3.32)	27.59 (1.86)	32.32 (0.37)
FSa	36.30 (1.60)	35.54 (0.65)	36.90 (1.84)	33.62 (0.91)	37.24 (3.17)	33.38 (2.72)	34.46 (1.40)
CSi	* 48.85 (1.85)	41.75 (2.15)	45.37 (1.60)	43.63 (2.28)	45.49 (6.18)	40.16 (3.45)	42.64 (1.54)
FSi+C	45.26 (2.96)	44.10 (2.44)	43.48 (3.68)	40.65 (1.64)	32.78 (4.30)	31.00 (2.57)	34.90 (1.47)
Shrubland							
CSa	36.01 (2.59)	b 31.53 (0.67)	a 27.18 (1.80)	b 31.40 (0.63)	b 29.03 (1.59)	a 22.99 (1.16)	b 29.16 (0.92)
FSa	35.11 (2.56)	b 39.34 (1.97)	a 32.56 (0.70)	a 33.26 (0.83)	b 36.00 (1.91)	a 26.63 (0.96)	a 29.72 (1.35)
CSi	44.56 (4.71)	b 48.06 (2.94)	a 39.89 (1.10)	a 38.94 (1.26)	b 42.62 (2.29)	a 30.59 (1.42)	a 33.61 (1.08)
Fsi+C	47.54 (3.33)	b 45.01 (1.13)	a 39.89 (1.27)	a 37.54 (1.01)	29.07 (1.67)	25.28 (1.52)	27.83 (0.66)

among fractions, time (day 3, year 6 and year 12 post-fires) and also between burned (day 3 post-fire) and unburned plots (day 3 post-fire) in distribution of N in fractions, N content, ^{15}N recovery, RIN, RI^{15}N , C:N ratio and recalcitrant C:N ratio. Duncan multiple range comparison test was applied when the significance level reached $p \leq 0.05$.

Results

Total N and RIN in fractions

Soil N was mostly located in the finest fraction—fine silt+clay (FSi+C)—with values between 50 and 60% of total N in all plant communities, without differences all over the experiment (Fig. 1). In contrast, the distribution of soil N after the fires in the other fractions was different depending on the plant community. In grassland no effects in total N after fire was observed at any fraction. In contrast, in shrubland and mixed shrub-grassland total N was higher in fine sand (FSa) and coarse silt (CSi) fractions 3 days post-fire and remained until year 12 post-fire. Coarse fraction (CSa) behaved in a different way. No changes or even a small decrease (shrubland, $p < 0.1$) in total N were found in coarse sand (CSa) fraction just after fires, whereas it increased from 6 years to 12 years post-fire in the shrubland and mixed shrub-grassland.

The recalcitrance of N (RIN) was higher in the fine fractions (CSi and FSi+C) than in the coarse fractions (CSa and FSa) (Table 1). In general, no differences existed just after fires between burned and unburned plots, except the decrease observed in CSi fraction of mixed shrub-grassland ($p < 0.05$) and grassland ($p < 0.1$). In the next 12 years, we did not detect significant changes in the RIN in any fraction, in the grassland and mixed shrub-grassland plots. In contrast, in the shrubland we observed a decrease in RIN of all fractions from day 3 to year 6 post-fire; after 12 years only coarse sand (CSa) fraction recovered the initial values.

C:N ratio in soil fractions ranged from very low values in FSi+C to quite high ones in the coarse fractions (Fig. 2). In general C:N ratio in fine fractions (CSi and FSi+C) did not show any change after the fires and only grassland showed higher C:N ratio in unburned FSi+C than in the burned plots. C:N ratio of coarse fractions was

lower in burned plots immediately after the fires compared with the unburned plots in all three communities. After fires, C:N ratio of coarse fractions did not change over time in shrubland whereas it increased in grassland and mixed shrub-grassland communities.

Recalcitrant C:N ratios were much higher than C:N ratio of the respective fraction (Fig. 2). This difference was especially large in coarse fractions. Just after fires, recalcitrant C:N ratio in CSa fraction was lower in mixed shrub-grassland and in shrubland in burned plots compared with control ones while no differences were detected in grassland. In the time after fire, recalcitrant C:N ratio increased in coarse fractions in grassland and mixed-shrubland (only in the CSa) while did not change in the shrubland. Only in the mixed shrub-grassland these changes over time on recalcitrant C:N ratio also occurred in the finest fraction (FSi+C).

Recovery of ^{15}N and RI^{15}N in fractions

^{15}N -tracer recovered in soil particulated fractions just after the ^{15}N -labelling ranged from 22-53%. A great part of the $^{15}\text{N-NH}_4^+$ in soil was lost during the fractionation process most likely in the soluble fraction that was discarded. Just after the fires, a substantial fraction of the added ^{15}N -tracer was located in the finest fraction (FSi+C) in grassland and shrubland (25 and 33 % of ^{15}N recovery at day 3 post-fire, respectively). In the mixed shrub-grassland the figures were lower (14% at day 3 post-fire) (Fig. 3).

No significant differences in ^{15}N -recovery were found over the 12 years in any plant community after fires. However the behaviour of both legume-free plant communities (grassland and shrubland) was remarkably similar. Thus we repeated the analysis of the evolution with time of the ^{15}N -tracer in both plant communities altogether (Table 2): we observed a decrease of significant quantities of ^{15}N -tracer from day 3 to year 6 post-fire in CSa and in FSi+C fractions (i.e. from 28 to 10 % and from 37 to 16% in FSi+C fraction in grassland and shrubland respectively) whereas there was an increase in ^{15}N -recovery only in the CSa fraction in the year 12 post-fire.

An important proportion of ^{15}N -tracer was retained in the recalcitrant fraction of all fractions immediately after fire (Fig. 3, Table 1), higher in the grassland and shrubland than in the

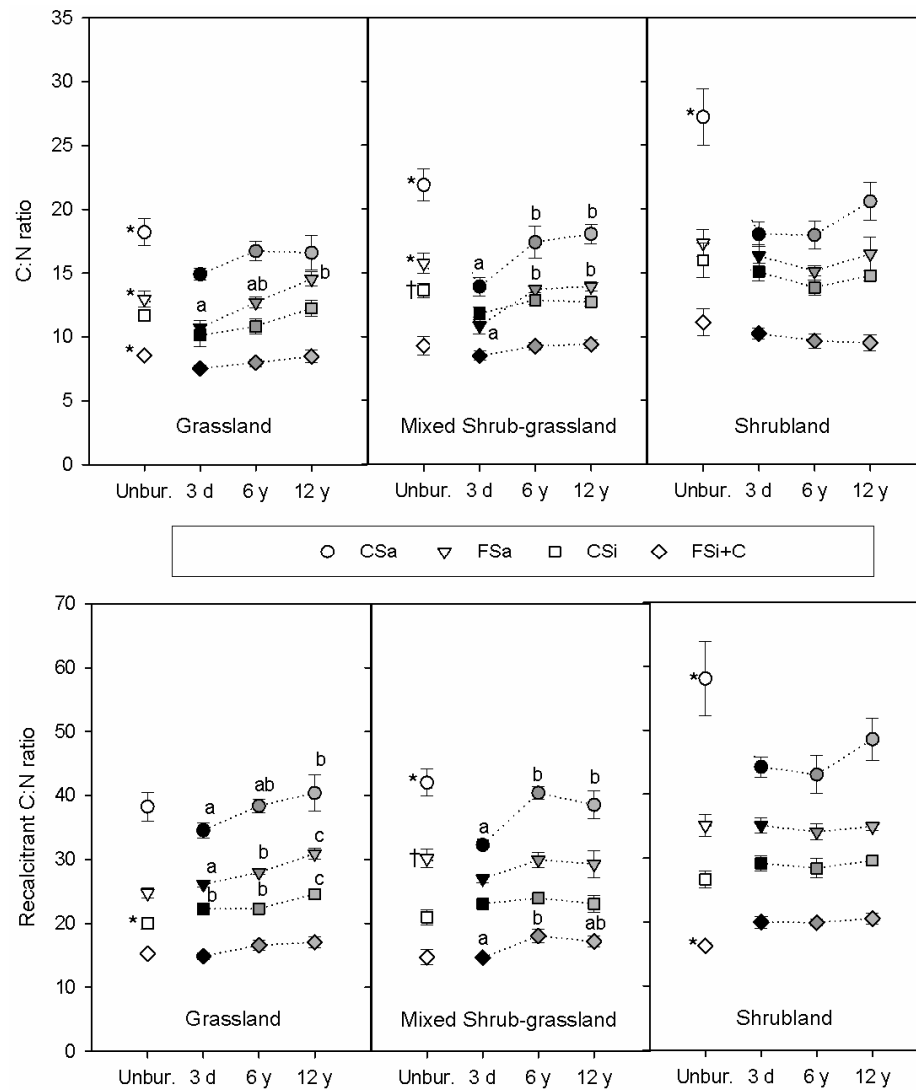


Fig. 2 C:N ratio and recalcitrant C:N ratio in fractions (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt+clay (FSi+C)) from unburned and burned soils (day 3, year 6 and year 12 post fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with † $p < 0.1$ and * $p < 0.05$. Within a given graph, time differences are labelled with a different lowercase letter, at $p < 0.05$. Values are means \pm SE ($n=5-6$)

mixed shrub-grassland (Fig. 3). In contrast, the highest $RI^{15}N$ was found in mixed shrub-grassland ($RI^{15}N$ between 33% and 45% at day 3 post-fire; Table 1). At day 3 for all plant communities the highest $RI^{15}N$ occurred in medium size fractions (FSa and CSi) while the lowest occurred in the coarsest (CSa) and finest (FSi+C) fractions. This retention pattern contrasted with the total N recalcitrance of this fraction (see RIN; Table 1).

The changes over time of the recovery of recalcitrant ^{15}N in each fraction showed different patterns depending on the plant community (Fig.

3). While no differences over time were found in mixed shrub-grassland, analyzing grassland and shrubland together we observed that recovery of ^{15}N in the recalcitrant fraction decreased from day 3 to year 6 post-fire in all fractions but, after that, the values increased again in CSa and CSi fractions (Table 2). From day 3 to year 6 post-fire, only shrubland showed decreases in recalcitrance ($RI^{15}N$) in all fractions except in the finest fraction (FSi+C) (Table 1). Similarly to RIN values, after the year 6 post-fire, the coarse fraction (CSa) was the only fraction

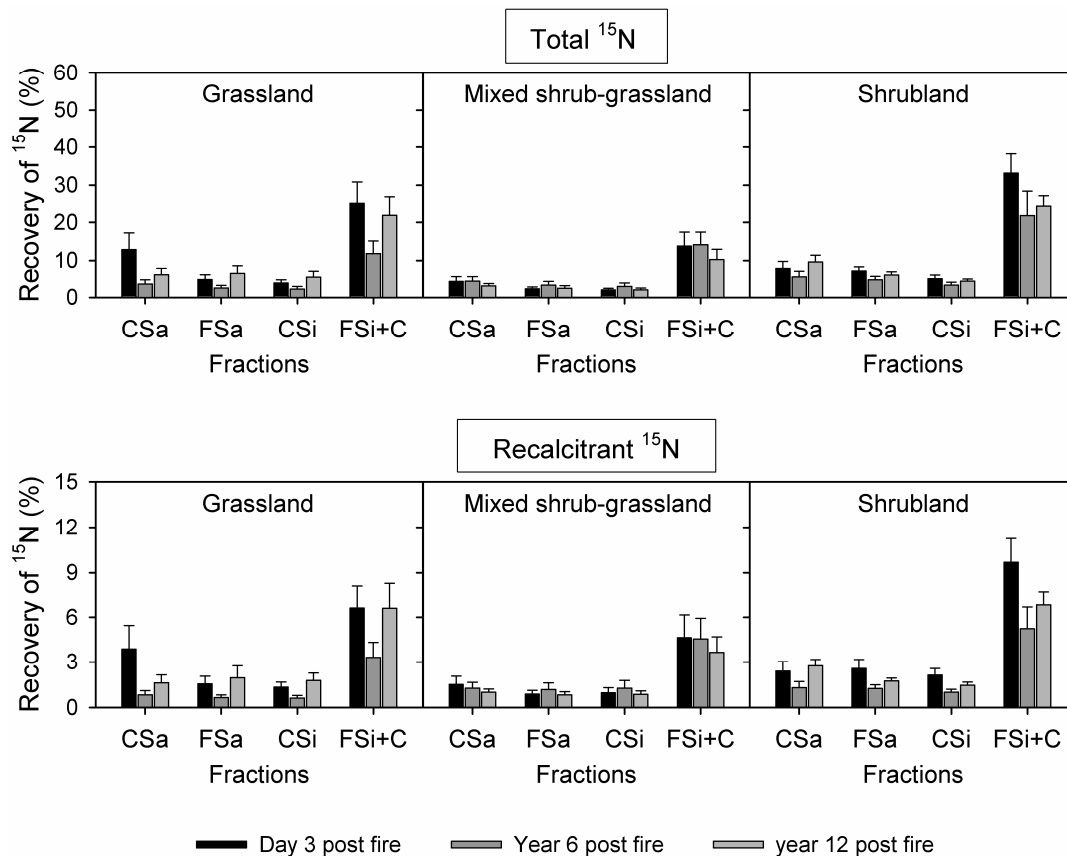


Fig. 3 Recovery of ^{15}N in soil (%) in fractions (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt+clay (FSi+C)) from burned soils (day 3, year 6 and year 12 post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Values are means \pm SE (n=5-6)

which increased RI^{15}N to similar values to 3 days post-fire.

Discussion

Changes in total N

Fire promoted a redistribution of N in the soil fractions mainly in woody plant communities (mixed shrub-grassland and shrubland). Fire has a shredding effect on plant litter (Scharenbroch et al. 2012). Thus, after fires N increased in medium size fractions (FSa, CSi) and remained over the whole studied period. This effect was only detectable in woody plant communities with higher biomass and with higher fire intensity (Romanyà et al. 2001). In contrast, N in the coarsest fraction (CSa) did not change shortly after fire or even showed lower values compared with unburned plots in the shrubland, which may be related with highest temperatures reached at the soil surface in this community. C:N ratio in the coarse fractions was much lower

in the burned plots, indicating large losses of organic C and therefore changes in organic matter quality after fire. The lack of net changes in total N observed right after the fire was likely the result for the compensation between losses by volatilization and the incorporation of new N in ashes and partly charred plant residues. In the burned woody communities, the total N in the CSa increased towards the end of sampling period, at the 12 year. This pattern may be related with an increase of organic matter deposition due to a drought period between the year 6 and 12, as described previously Martí-Roura et al. (2011). Thus, the N originated during or shortly post-fire was allocated in medium particles (FSa and CSi) while N resulting from a severe drought, mostly particulated, was mainly held in the coarsest fraction (CSa) and was likely originated from dead coarse root deposition.

In our experiment N recalcitrance did not change after fire in any of the soil fractions. In coarse fractions of burned woody plant communities recalcitrant C:N ratio was lower

than unburned plots, indicating that values of recalcitrant C were lower after fire. These findings are in contrast to those of Rovira et al. (2012) that showed increases in N and C recalcitrance post-fire in the bulk soil. In the first years following fire N recalcitrance decreased only in shrubland, the only plant community dominated by seeders. Seedling litter deposition was enriched in non woody plant material as compared to that of mature shrubs. This would have contributed to the increase of the labile N fraction in this plant community.

As expected, most N was found in the finest fraction (FSi+C) and, in agreement with Rovira et al. (2010), this fraction and the fine silt fraction showed higher recalcitrance than coarse fractions (Table 1). Unlike in the coarse fractions, the stabilization of organic matter in fine fractions is not only controlled by the inherent molecular-level resistance to microbial breakdown, but also by the association of organic matter with aggregates or minerals (Christensen 2001; Swanston et al. 2002).

Recovery of ^{15}N in soil fractions

The greatest proportion of ^{15}N -tracer applied after the fire was quickly retained in fine fractions $<20\ \mu\text{m}$ (FSi+C). Hilscher and Knicker (2011) also observed that most of the ^{15}N -tracer applied after a burning process was found in the finest fraction ($<2\ \mu\text{m}$ fraction). This could be a result of $^{15}\text{N-NH}_4^+$ quick sorption into the cation exchange complex. However, an important part

of the ^{15}N -tracer was also retained in the coarsest fraction. As the coarse organic debris are believed to be the main C source for soil microflora (Hagedorn et al. 2003; Rovira et al. 2010) we suggest that $^{15}\text{N-NH}_4^+$ could be quickly retained in this fraction through microbial processes incorporating part of the ^{15}N -tracer into this particulate organic matter fraction.

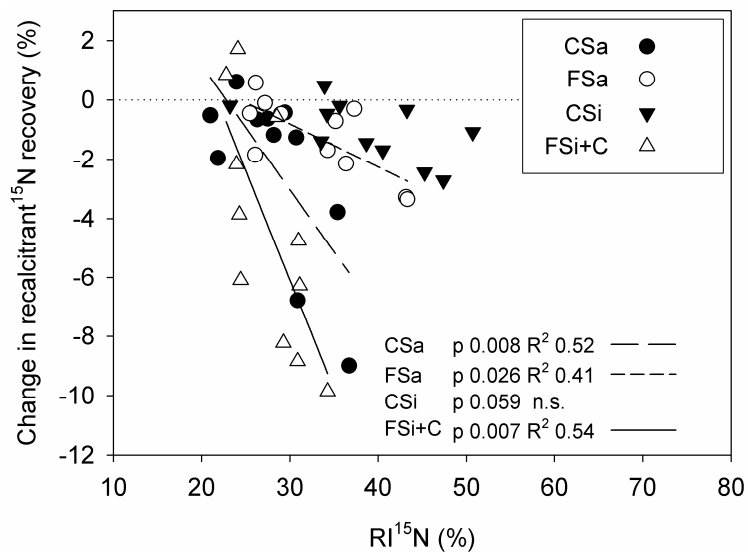
As expected, this newly incorporated ^{15}N -tracer was less recalcitrant (RI^{15}N) than the total N (RIN) suggesting that in case of microbial accessibility this N pool would be more prone to biological transformations than the older N in the fraction. Knicker and Skjemstad (2000) found the presence of peptide-like structures, N-rich particles traditionally considered chemically and physically labile fractions of soil organic matter, in residues of fine soil fractions ($<53\ \mu\text{m}$). The presence of these N-rich compounds in fine fractions could lower the recalcitrance of such fraction that, in our case, was indeed less recalcitrant than FSa and CSi. Garcia-Pausas et al. (2012) found a rapid incorporation of less recalcitrant compounds in the finest fraction (FSi+C) that were especially rich in N, likely coming from microbial resynthesis (Rumpel et al. 2010; von Lützow et al. 2006). In our experiment, low recalcitrance of newly incorporated ^{15}N -tracer was found in both the finest (FSi+C) and the coarsest fraction (CSa) but not in the intermediate fractions.

Thus, as we could observe in figure 4, during the first 6 years post-fire, the changes of the recalcitrant ^{15}N -tracer in most particle size

Table 2. Changes over time (day 3, year 6 and year 12 post-fire) on the total and recalcitrant ^{15}N recovery in grassland and shrubland analyzed together showing the differences on time for each soil fraction (coarse sand (CSa), fine sand (FSa), coarse silt (CSi), fine silt+clay (FSi+C)). Time differences are labelled with a different lowercase letter, at $p<0.05$.

	Time post-fire			<i>P</i> -value
	3 days	6 years	12 years	
<i>Recovery of total ^{15}N</i>				
CSa	10.40 (2.39) b	4.43 (0.92) a	8.03 (1.35) b	0.05
FSa	5.91 (0.96)	3.50 (0.66)	6.15 (1.05)	0.10
CSi	4.42 (0.63)	2.75 (0.49)	4.83 (0.73)	0.06
FSi+C	29.21 (3.81) b	16.36 (3.67) a	23.17 (2.68) ab	0.04
<i>Recovery of recalcitrant ^{15}N</i>				
CSa	3.14 (0.84) b	1.05 (0.24) a	2.24 (0.37) b	0.02
FSa	2.06 (0.41) b	0.92 (0.17) a	1.84 (0.36) ab	0.05
CSi	1.74 (0.29) b	0.79 (0.14) a	1.60 (0.24) b	0.01
FSi+C	8.14 (1.15) b	4.19 (0.87) a	6.72 (0.88) ab	0.03

Fig. 4 Relationship between $RI^{15}N$ (%) at day 3 post-fire and the changes in recalcitrant ^{15}N recovery (%) between 6 year and 3 days post-fires in each soil fraction (coarse sand (CSa), fine sand (FSa), coarse silt (CSi) and fine silt+clay (FSi+C))



fractions negatively related to its initial recalcitrance ($RI^{15}N$) (day 3 post-fire). The steepest slopes of these relationships were observed for the finest and coarsest fractions (FSi+C and CSa) while the medium size fractions with higher recalcitrance showed much lower slopes or non significant relationships. This suggested high sensitivity of changes in recalcitrant ^{15}N pool to the substrate recalcitrance occurring in these two fractions. As mentioned above the finest and coarsest fractions showed the most prominent changes over time of total ^{15}N recovery indicating that recalcitrant ^{15}N in these fractions was more readily transformed than in the medium size particle fractions (FSa and CSi).

Plant communities free of legume shrubs showed over time decreases of the ^{15}N -tracer pool that affected both the coarsest (CSa) and the finest (FSi+C) fractions. It appears therefore that the physical protection by the fine particles did not stop the ^{15}N -tracer from being exported from this fraction. Interestingly, these over time changes affected to all soil fractions, when looking at the recalcitrant ^{15}N . This suggests that the ^{15}N -tracer transformations in the medium size fractions (FSa and CSi) mainly involved the recalcitrant fraction while in the FSi+C and CSa fraction involved the whole pool (labile and recalcitrant) of ^{15}N and coincided with a specially low ^{15}N recalcitrance at day 3 post-fire (Table 1).

In contrast the retention of ^{15}N -tracer did not show any change over time for the mixed shrub-grassland. It is interesting to note that this plant community showed the highest $RI^{15}N$ for all soil fractions only 3 days after labelling. Overall N recalcitrance was quite similar among all

vegetation types but, unlike the other vegetations, mixed shrub-grasslands readily seemed to incorporate ^{15}N -tracer in more N recalcitrant pools. These processes may be influenced by the specificity of the soil microbial communities occurring in plant communities with mixed legumes (Ladygina and Hedlund 2010; Zhou et al. 2012). From our data set we can infer that the retention of the ^{15}N -tracer even in the fine fraction (FSi+C), during the years following fire, was mainly regulated by the recalcitrance of the substrate rather than the physical protection. Consequently, differences in soil organic matter quality i.e. changes in vegetation type seemed to lead the fate of the ^{15}N -tracer in soil after fires.

The observed drought-induced total N increases (Martí-Roura et al. 2013), an increase of ^{15}N -tracer were observed between the year 6 and 12 post-fire mainly in the coarsest fraction (CSa) but only in legume-free plant communities. Increases in finer fractions were not significant. This indicates that N in root deposition during drought periods is partly held in coarser debris.

Conclusions

Fire promotes changes in N cycle mainly affecting two different processes: (1) changes of soil N distribution in different size fractions, increasing N associated to medium size fractions due to new inputs of particulated charred material that will be readily retained in the following years; (2) fast incorporation of soluble mineral N in both the fine fraction and the

coarsest fraction likely as a consequence of an increased N mineralization after fire. The ^{15}N -tracer incorporated in these fractions (finest and coarsest) resulted to be more labile N than N hold in medium size fractions. As a result, N transformations in the coarsest and finest fractions (CSa and FSi+C) were greater than in medium size fractions (FSa and CSi) and affected both labile and recalcitrant ^{15}N forms.

Our data suggest that physically protected soil environments hold a relevant part of the N transformations. These transformations can affect both labile and recalcitrant N forms. Differences in recalcitrance or on microbial contexts associated to the chemical quality of the plant debris may control the mineralisation of N in both physically protected and unprotected environments.

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Capítol 4:

Dinàmica de la matèria orgànica pirogènica després del foc en prats i matollars mediterranis

Chapter 4:

Pyrogenic organic matter dynamics after fire in Mediterranean grassland and shrubland

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Resum capítol 4

En els incendis forestals, la combustió incompleta de la matèria orgànica origina estructures orgàniques altament condensades generalment anomenades matèria orgànica pirogènica (MOPi). Compostos de C i N refractaris són els principals constituents de la MOPi i una ràpida i persistent retenció de N mineral pot tenir lloc en aquesta fracció mitjançant interaccions als nuclis aromàtics. Els objectius del present estudi van ser determinar (1) els canvis en la qualitat i la quantitat de la MOPi induïts per focs experimentals que van tenir lloc a matollars i prats mediterranis, (2) els canvis en aquesta MOPi que van tenir lloc al llarg de 12 anys després dels incendis, i (3) quantificar l' incorporació i l'estabilització de la reserva de N mineral a la fracció més refractària. Una sèrie de focs experimentals es van dur a terme a tres comunitats vegetals. Un dia després dels focs, vam seleccionar una subparcel·la dins de cada parcel·la cremada i vam marcar la reserva de N mineral amb $^{15}\text{N-NH}_4^+$ (99 atm %). Posteriorment, es va mostrejar de 0-5 cm de sòl 3 dies, 6 anys i 12 anys després del foc i els sòls van ser sotmesos a una oxidació amb dicromat potàssic a baixa temperatura per obtenir el C orgànic elemental resistent (COER), el N (NOER) i el ^{15}N ($^{15}\text{NOER}$). El COER d'una comunitat vegetal representativa va ser analitzada per espectroscopia de ressonància magnètica nuclear de ^{13}C en fase sòlida. La combinació d'aquestes tècniques ens va permetre observar que tots els focs van fer augmentar el COER i el NOER independentment de la intensitat del foc, però només les brolles van mostrar canvis al llarg del temps coincidint amb un gran augment de la matèria orgànica al sòl que va ser associat a un període de sequera. Aproximadament entre 10-14 % de la recuperació del ^{15}N va ser ràpidament retinguda a la MOPi indicant que una proporció important del reservori de N mineral després del foc pot ser immediatament retingut en estructures de la MOS altament refractàries. A brolles i prats aquest ^{15}N retingut a la fracció de MOPi es va mantenir força estable durant els següents 12 anys després del foc però va disminuir en els prats emmatats a partir del dia 3 fins a l'any 12 després dels focs.

Black C and black N dynamics after fire in Mediterranean grassland and shrubland

Mireia Martí-Roura • Pere Rovira • Pere Casals • Joan Romanyà

Abstract

In forest fires, incomplete combustion of organic matter originates highly condensed organic structures generically called pyrogenic organic matter (PyOM). Organic C and N of the PyOM are defined as black C and black N. A fast and persistent retention of mineral N in this refractory fraction of soil organic matter may occur through interactions to the aromatic nuclei. In many studies black C and black N have been measured as 'organic resistant elemental C (OREC) and N (OREN)'. The objectives of the present study were to determine (1) the changes in OREC and OREN composition and quantity induced by experimental fires in Mediterranean shrubland and grassland, (2) to monitor the changes in OREC and OREN occurring over the following 12 years post-fire, and (3) to quantify the incorporation and the stabilization of the post-fire mineral N pool in the PyOM fraction. In three Mediterranean plant communities we set a series of experimental fires. One day after the

fires, we selected a subplot inside each burned plot and we labelled the mineral N pool with $^{15}\text{N-NH}_4^+$ (99 atom %). We collected 0-5 cm soil samples 3 days, 6 years and 12 years post-fire and soils were subjected to a low temperature dichromate oxidation to get OREC, OREN and ORE ^{15}N . The OREC of a representative plant community was characterized by solid-state ^{13}C NMR spectroscopy. The combination of these techniques allowed us to observe that all fires increased both OREC and OREN irrespective of the fire intensity but only shrubland showed over time dynamics coinciding with a large increase of soil organic matter that was associated to an extended drought period. About 10-14 % of the ^{15}N -recovery was quickly retained in the PyOM indicating that a significant proportion of the post-fire mineral N pool can be readily retained in the highly refractory structures of SOM. In shrubland and grassland this ^{15}N -tracer retained in the PyOM fraction remained quite stable during the following 12 years post-fire but decreased in mixed shrub-grassland from day 3 to year 12 post-fire. Thus changes on the refractory organic matter pool are not always associated to fire as they can also respond to large inputs of organic matter occurring several years after fire. N incorporated into this fraction can be remobilized over a period of few years.

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Keywords Prescribed fire • Oxidation resistant elemental carbon • ^{15}N -recovery

Introduction

Soil organic matter (SOM) in Mediterranean forest soils is highly subjected to changes due to the high recurrence of forest fires in that area. Quantity and quality changes of SOM after fires are strongly related to characteristics of fire and plant biomass and physical properties of soil (Certini 2005; Knicker 2007). After fire, incomplete combustion processes can induce severe chemical transformations in SOM. This promotes the formation of pyrogenic organic matter (PyOM) that is mainly composed of highly condensed aromatic structures highly resistant to degradation. These structures are made of a broad continuum of carbon types which range from residues derived from the incomplete combustion of plant tissues to inert graphitic carbon (Gélinas et al. 2001). C retained in these structures is generally called black C likewise its N is called black N. The chemical complexity and variety of black C drove to the co-existence of a great variety of methods (e.g. UV-oxidation, quantification of specific biomarkers like benzene-polycarboxylic acids) that can considerably vary the results obtained. One of the most widely used methods for the detection of black C, is the carbon fraction resistant to chemical oxidation with dichromate (Bird and Gröcke 1997). This method analyses a residue corresponding to the biologically and chemically refractory organic matter. Lately, complementary analysis such as H/C and O/C ratio and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy have revealed that black C fraction includes other 'non-black C compounds' highly resistant to chemical oxidation (Hammes et al. 2007; Knicker 2007). In consequence recent studies have adapted the term 'organic resistant elemental C (OREC)' for this highly resistant fraction (e.g. Alexis et al. (2012); Rivas et al. (2012)).

Black C in soils is generally assumed to be one of the most stable SOM compounds with a high degree of inertness for biological and chemical reactions. Lately, the understanding of stability of black C in soils has been considered crucial in order to balance the global C budget in a climate change context (Czimczik et al. 2005). However, there is increasing evidences that black C is more susceptible to losses than it was thought (Bird et al. 1999; Rovira et al. 2009). Several mechanisms like black C oxidation by

subsequent fires, slow chemical oxidation and solubilization of soil black C and black C susceptibility to biological degradation can account for black C loss over long-time scales (Preston and Schmidt 2006; Rovira et al. 2009).

Fires can provoke the transformation of N forms into pyrogenic N-heterocyclic structures—black N—which are incorporated to the microbiologically less accessible SOM pool (Knicker et al. 1996; Knicker 2010). Indeed, charcoal properties such as large surface area, negative surface charge, and high charge density, confer to this pyrogenic material high ion sorption capacity (Liang et al. 2006). Some mineral N forms (basically NH_4^+) can be readily retained in the black N pool by immobilization in heterocyclic structures (Ding et al. 2010; Yao et al. 2012) contributing to N stabilization in soils and reducing post-fire N leaching. Despite in most black C models black N is neglected, black N might also play an important role in the stabilization of SOM and N recycling in fire-affected regions. The conception of PyOM not only as aromatic C-rich compound but as a heterogeneous mixture of thermally altered macromolecules with N and O substitutions (Knicker et al. 2008; Knicker 2007) has also suggested that, in fact, PyOM may have lower biological and chemical stability compared to traditional assumptions usually made in black C models (Gårdenäs et al. 2011). Thus, we hypothesize that stability of the refractory organic matter pool in burned soils can be affected by the soil organic matter quality and N dynamics under contrasted vegetation types. In this context, ^{15}N labelling field experiments were performed using enriched $^{15}\text{N-NH}_4^+$ to label the mineral pool after the fires and analyze the incorporation of this newly deposited N to the PyOM fraction and its persistence within the fraction. Thus, we aimed to study (1) the changes in OREC and OREN composition and quantity induced by experimental fires in Mediterranean shrubland and grassland, (2) the changes in OREC and OREN occurring over the following 12 years post-fire, and (3) quantify the incorporation and the stabilization of the post-fire mineral N pool in the PyOM fraction.

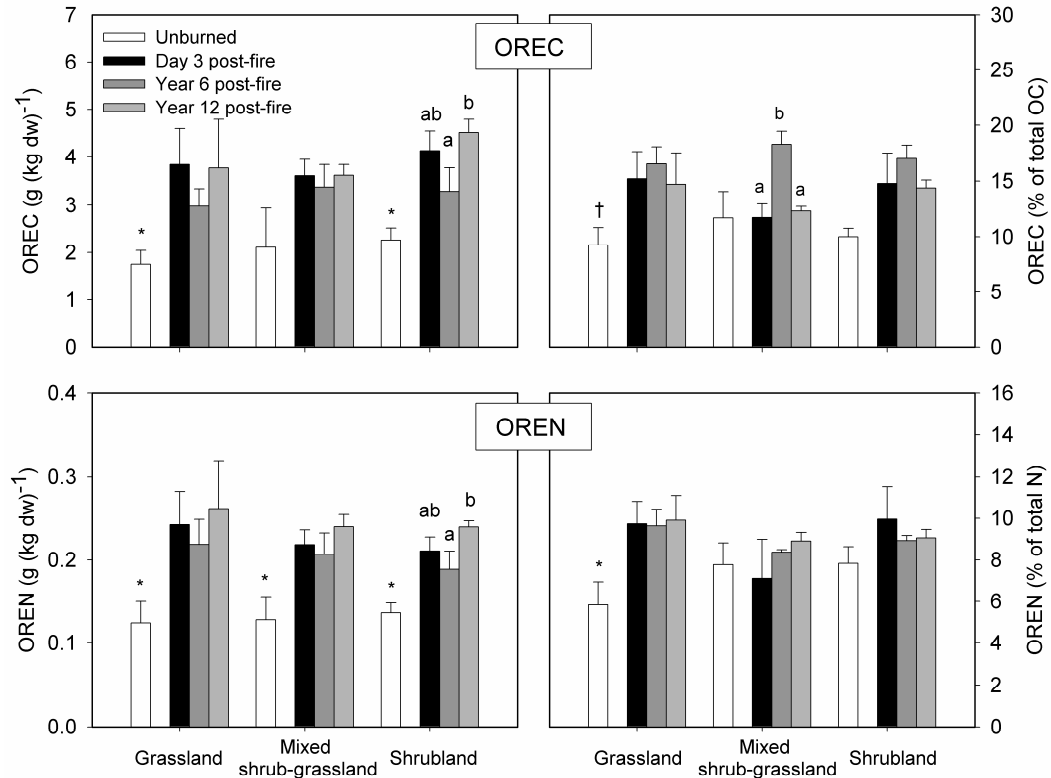


Fig. 1 Oxidation resistant elemental C (OREC) and N (OREN) in unburned and burned soils (3 days, 6 years and 12 years post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with † $p < 0.1$ and * $p < 0.05$. Within a given graph, time differences are labelled with a different lowercase letter, at $p < 0.05$. Values are means \pm SE ($n = 5-6$)

Material and methods

Study site

We selected three different plant communities located in abandoned fields in the NE of Iberian Peninsula ($41^{\circ} 56' N$, $0^{\circ} 37' E$, 460 m a.s.l.). The abandonment of the terraced fields was in early 1960s. The three different plant communities

representative of the Mediterranean land set aside dominating the old fields were: grassland dominated by the perennial grass *Brachypodium retusum* (Pers.) Beauv; mixed shrub-grassland dominated by the shrub *Genista scorpius* L. in Lam et DC and the grass *B. retusum* and the shrubland dominated by *Rosmarinus officinalis* L. The climate is dry Mediterranean continental, the mean annual temperature is $13.5^{\circ}C$ and the

Table 1. C/N and H/C ratio in OREC of unburned and burned plots (3 days, 6 years and 12 years post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Differences between burned and unburned plots 3 days post-fire are labelled with * $p < 0.05$.

	C/N ratio				H/C ratio			
	Unburned	Burned			Unburned	Burned		
	3 days	3 days	6 years	12 years	3 days	3 days	6 years	12 years
Grassland	14.6 \pm 0.9	15.5 \pm 0.9	14.3 \pm 0.9	13.2 \pm 1.8	3.5 \pm 0.3	2.7 \pm 0.4	2.6 \pm 0.1	2.5 \pm 0.5
Mixed shrub-grassland	15.9 \pm 3.2	16.5 \pm 0.7	16.2 \pm 0.2	15.7 \pm 1.4	4.9 \pm 0.1*	2.5 \pm 0.3	2.9 \pm 0.3	2.2 \pm 0.4
Shrubland	17.1 \pm 2.1	19.7 \pm 1.6	17.1 \pm 0.7	16.9 \pm 1.5	3.8 \pm 0.4*	2.2 \pm 0.2	3.0 \pm 0.4	2.1 \pm 0.3

mean annual precipitation is 516.9 mm (observation period: 1996–2008; Monestir de les Avellanes, 41° 52' N, 0° 45' E, 580 m a.s.l.). The rainfall distribution is markedly seasonal, with maximum values in spring and autumn and a dry season that usually lasts from June to September. The soils are Calcaric Cambisol (FAO-UNESCO 1988), developed from a fine textured Eocene limestone and marl colluvium, with a pH (H₂O) of 8.2, 60% calcium carbonate content and the soil texture is loam. The study areas had been free of fire and grazing by domestic animals for at least 10 years before the beginning of the study.

Experimental design, Soil ¹⁵NH₄⁺ labelling and soil sampling

Six pairs of plots (from 20 to 60 m²) of each type of vegetation distributed in different terraces were selected (18 pairs of plots in total). In 1996 a plot of each pair was experimentally burned while the other remained as a control plot, representing the undisturbed state. Fire intensity was low to medium, with temperatures at ground level from 300 to 526°C. See Romanyà et al. (2001) for more details.

Three days after the fires, subplots of 2x2 m were bordered in each burned plot and a solution highly ¹⁵N-enriched (¹⁵NH₄Cl, 99 atom%, 1 kgN ha⁻¹) was applied on the ashes of these subplots. Subplots were divided in 64 squares (25x25 cm) and sprinkled with 18.75 ml of ¹⁵NH₄Cl solution (1 l m⁻² of 0.333 mg ¹⁵N l⁻¹) in order to homogenize ¹⁵N labelling on the subplots surface (see Casals et al. (2005) for more details).

From each plot, three soil cores (5x5 cm) from 0 to 5 cm depth were taken and bulked in a sample (n = 36). Samplings were carried out 3 days (just after labelling), 6 years and 12 years post-fire at ¹⁵N-labelled and burned plots and 3 days post-fire in unburned plots. Then soil samples were air-dried and sieved (2 mm).

Organic resistant elemental C (OREC) and N (OREN) analysis

The OREC and OREN fractions of SOM were quantified to assess the proportion of chemically resistant C and N after the fires by using a low-temperature dichromate oxidation (Masiello et al. 2002; Wolbach and Anders 1989). The protocol we used was a slight modification of Rumpel et al. (2006) performed by Rovira et al.

(2009). Briefly, we carried out a pretreatment with 10% HF. So we added 15 ml of 10% HF to 2 g of 2mm-sieved soil and it was shaken overnight. Then, it was centrifuged (3000 rpm) and the liquid was decanted and discarded. This procedure was repeated twice and finally the residue was washed with deionized water and dried at 60°C. After this first treatment, the second step with potassium dichromate reaction was carried out. We put 150 mg of HF-treated soil in a Pyrex tube and 20 ml of 0.25 M K₂Cr₂O₇ in 2 M H₂SO₄ solution was added. The tube was not hermetically sealed and submitted to a slight shaking for 1 week at room temperature. Then it was centrifuged and the liquid phase was discarded. The residue was washed with deionized water and centrifuged again to discard the liquid phase. Then, the dichromate oxidation treatment was repeated. Finally, the residue was washed with deionized water, centrifuged to eliminate the excess of water and dried at 60°C. This residue was analyzed for total C, N and δ¹⁵N with an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ratio mass spectrometer (IRMS) (20–20 isotope ratio mass spectrometer; PDZ Europa, Sercon Ltd., Cheshire, UK). H was analyzed at Scientific and Technological Services of the University of Barcelona using an elemental organic analyzer Thermo EA 1108 (Thermo Scientific, Milan, Italy).

To calculate the atom % ¹⁵N excess in each sampling we used the following formula:

$$\text{atom\% } ^{15}\text{N excess} = \text{atom\% } ^{15}\text{N}_L - \text{atom\% } ^{15}\text{N}_C \quad (1)$$

Where atom% ¹⁵N_L is the concentration of ¹⁵N-tracer in the labelled plots and atom% ¹⁵N_C is the concentration of ¹⁵N-tracer in the unlabelled (burned-control) plots.

To estimate the proportion of ¹⁵N-tracer that was located in the black N pool, we used the following mass-balance equation:

$$\begin{aligned} &\% \text{ Recovery of } ^{15}\text{N} \\ &= \frac{\text{atom\% } ^{15}\text{N excess}_t * N_t}{\text{atom\% } ^{15}\text{N excess}_0 * N_0} \times 100 \quad (2) \end{aligned}$$

N_t and N₀ are the total amount of N in soils (mg N kg⁻¹) at time *t* and time 0.

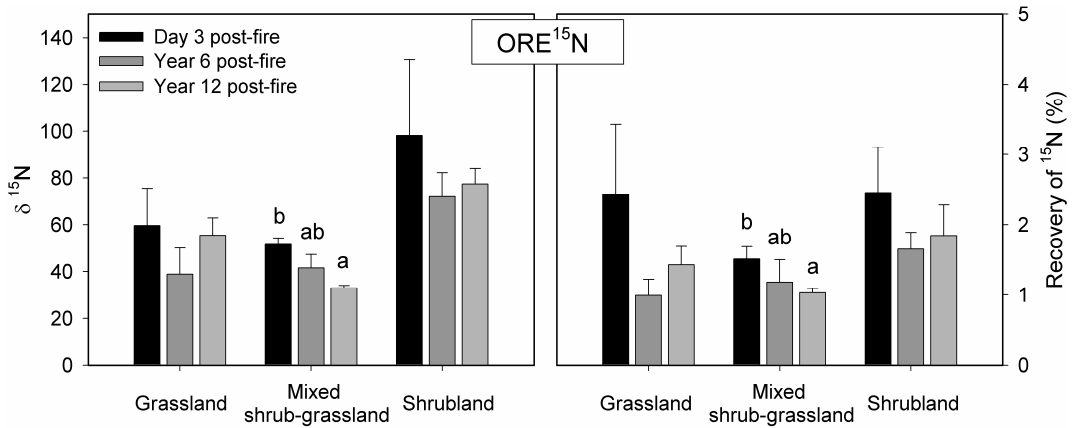


Fig. 2 Oxidation resistant elemental ^{15}N (ORE^{15}N) in burned soils (3 days, 6 years and 12 years post-fire) in three different plant communities (grassland, mixed shrub-grassland and shrubland). Time differences are labelled with a different lowercase letter, at $p < 0.05$. Values are means \pm SE ($n=5-6$)

Solid-state ^{13}C NMR spectroscopy

To know about the composition of the resistant residue we selected the oxidated samples of the shrubland soils corresponding to each one of the sampling times before and after the fire (unburned and 3 days, 6 years and 12 years post-fire). The 6 replicates per time were bulked to a single sample and analyzed by solid-state ^{13}C NMR spectroscopy using a Bruker Avance 600 MHz Wideboard operating at a frequency of 150.93 MHz with zirconium rotors of 4 mm OD with KEL-F-caps. The cross polarization magic angle spinning (CPMAS) technique was applied during magic-angle spinning of the rotor at 14 kHz. A ramped ^1H -pulse was used during a contact time of in order to circumvent spin modulation of Hartmann-Hahn conditions. A contact time of 1 ms and a 90° ^1H -pulse width of

2.4 μs were used for all spectra. The ^{13}C -chemical shifts were calibrated to tetramethylsilane (= 0 ppm) and were calibrated with glycine (176.04 ppm). The total abundances of the various C-groups were determined by integration of the signal intensity in their respective chemical regions according to Knicker et al. (1996).

Statistical analysis

We used a General Linear Model (GLM)-Repeated Measures analysis to test the effects of time on OREC, OREN, $\delta^{15}\text{N}$, ^{15}N -recovery, C/N and H/C in soil for each community. Our data were normally distributed and we used Levene's test to check homogeneity of variances before statistical analysis. When variances were unequal, original data were log-normal

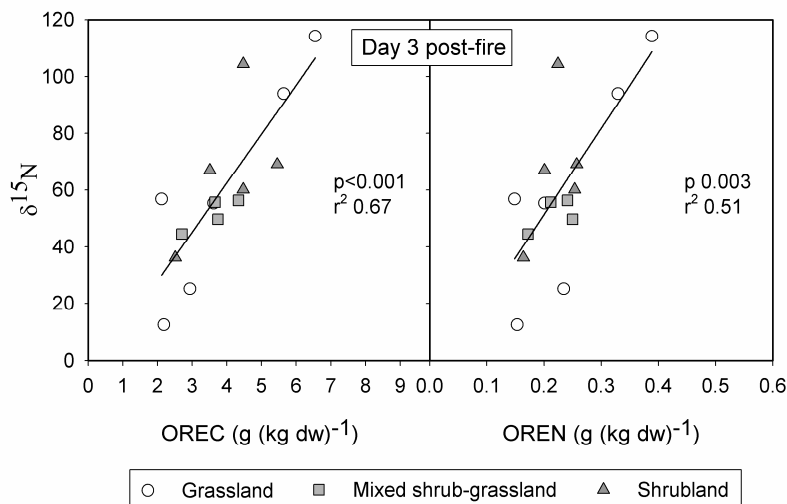


Fig. 3 Relationship between $\delta^{15}\text{N}$ (‰) and OREC and OREN at day 3 post-fire

transformed. As the data did not fail the sphericity test, the assumed sphericity correction was used. We used the Bonferroni method to test the significance of multiple comparisons. The probability threshold used to determine significance was $p < 0.05$.

We also used a One-Way Analysis of Variance (ANOVA) to test differences OREC, OREN, C/N ratio and H/C ratio between burned and unburned plots. Duncan multiple range comparisons were applied for each significant factor.

Results

OREC and OREN in soils

OREC represented between 9 and 18% of the total organic C and OREN represented between 6 and 10% of the total N (Fig. 1). After fires, OREC and OREN content increased in all plant communities. These increases ranged from 71 to 119% in OREC and from 55 to 91% in OREN and the largest differences between unburned and burned plots were found in grasslands. These increases were not only associated to increases in OREC and OREN but also to the TOC and total N as no significant differences were observed in OREC and OREN as % of total organic C and % of total N between unburned and burned plots (Fig. 1). The over time dynamics depended upon the vegetation. Grassland and mixed shrub-grassland did not show any change over the 12-year post-fire period and only shrubland showed increases in OREC and OREN content between year 6 and 12 post-fire. The percentage of OREC referred to TOC only showed a high value of OREC at year 6 post-fire in mixed-shrubland that coincided with a large decrease in TOC. In table 1 we can observe that C/N ratio did not change after fire, but this was not the case for H/C ratio that showed a decrease in mixed shrub-grassland and shrubland. In our study H/C ratio values, in addition to the pyrogenic materials, included the mineral matter which contains high quantity of H and very little or no C. We assumed that mineral background did not change among samples and thus the changes in H/C ratio were attributed to changes in the residual organic material.

In the top 5 cm of soil between 2 and 3% of the ^{15}N -tracer were directly retained in the

charcoal fraction 3 days post-fire (Fig. 2). These findings were also supported by the positive correlations observed between ^{15}N -tracer and both OREC ($r=0.82$) and OREN soil content ($r=0.71$) just after the fires (Fig. 3). While no differences were observed neither in ^{15}N -tracer enrichment or ^{15}N -recovery in grassland and shrubland over time, mixed shrub-grassland decreased both parameters over 12 years post-fire (Fig. 2).

Solid-state ^{13}C NMR

Soils of shrubland communities showed significant changes in OREC and OREN over-time (Fig. 1). In order to know whether these changes occurred along with the biochemical composition of these recalcitrant residues we analyzed them for solid-state ^{13}C NMR (Fig. 4 and Table 2). Overall, we could observe that O-

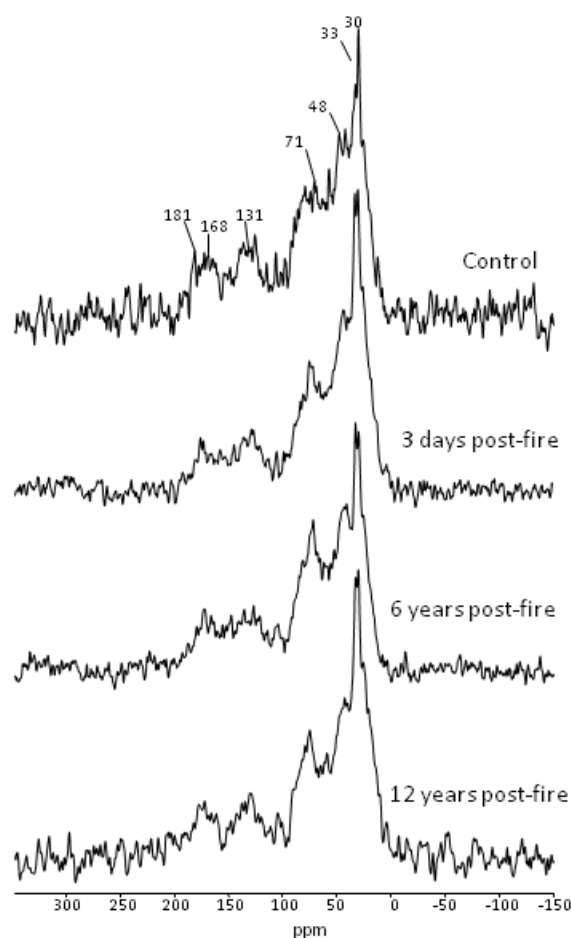


Fig. 4 Example of solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) spectra of burned shrubland after demineralization with HF and dichromate oxidation at 0-5 cm depth

Table 2. ^{13}C NMR for chemical shift^a assignments for burned shrubland after demineralization with HF and dichromate oxidation at 0-5 cm depth

	Carboxyl C	Aryl C	O-Alkyl C	Alkyl C	Total
<i>Control</i>					
^{13}C -intensity (%)	12.0	15.4	39.7	32.4	100
% of Corg	1.2	1.5	4.0	3.2	10
<i>3 days post-fire</i>					
^{13}C -intensity (%)	7.1	13.7	37.0	42.1	100
% of Corg	1.0	2.0	5.5	6.2	15
<i>6 years post-fire</i>					
^{13}C -intensity (%)	10.8	15.4	40.7	32.5	99
% of Corg	1.8	2.6	6.9	5.5	17
<i>12 years post-fire</i>					
^{13}C -intensity (%)	12.3	12.6	34.5	39.7	99
% of Corg	1.8	1.8	5.0	5.7	14

^a Carboxyl (160-220 ppm), Aryl C (110-160 ppm), O-alkyl C (45-110ppm) and Alkyl C (0-45ppm)

alkyl C and alkyl C were the most abundant compounds representing between 72 and 79% of the variation in the spectra and amounting between 7 and 12% of the TOC in soils (Fig. 4, table 2). Furthermore alkyl C showed a large variation over time with higher values 3 days and 12 years post-fire, coinciding with peaks of organic matter deposition. Over time changes in O-alkyl C were much smaller. On the other hand, a small contribution of carboxyl C and aryl C regions were observed and no big changes of these compounds were detected after fire.

Discussion

Changes and quality of the OREC fraction

In this study OREC can account for an important fraction of SOM ranging from 9 to 18%. Other studies using similar oxidation methods showed much lower levels normally below 10% independently of the climate (e.g, subtropical soils, Alexis et al. (2012); Mediterranean soils, Rovira et al. (2009); tropical soils, Rumpel et al. (2006)). Fire can increase refractory C and N due to the neoformation of condensed aromatic

structures during this disturbance (Almendros et al. 2003; Knicker et al. 1996), but surprisingly these increases usually are not translated in increases of OREC and/or OREN (Rivas et al. 2012; Rovira et al. 2009). In our case, the clearer increases occurred in the lowest intensity fires, such as those of grasslands (Romanyà et al. 2001), in which both OREC and OREN increased independently of the TOC and total N content. On the other hand, Rivas et al. (2012) observed that the aromatic region of OREC (COREC_{arom})—considered an estimation of black C fraction— only represented a small percentage of TOC (from 0.5% in unburned forests to 6.9% in high severity fires), although they found large increases of this aromatic region in burned forest soils. Accordingly to these findings, the solid-state ^{13}C NMR results in our study indicated that the aromatic region (aryl C) in shrubland communities had also a small contribution to the TOC and a slight tendency to increase during the first 6 years post-fire (from 1.5% in unburned plots to 2.6% in burned plots 6 years post-fire). The decrease of H/C ratio after fires in shrubland and mixed shrub-grassland also suggested the accumulation of aromatic compounds 3 days after fire. In contrast, grassland in spite of

showing the highest OREC and OREN increases, doubling the unburned values after the fires, their H/C ratio did not change. This may indicate even less abundance of aromatic compounds in the OREC fraction. It has been stated that the differences in H/C ratio in PyOM depend on the type of plant material and the intensity of heating (Hammes et al. 2006).

While aromatic compounds only represented a small fraction in OREC after the fires, aliphatic compounds (alkyl C) were the most abundant structures in the resistant residue of shrubland in the top 5 cm. Lipids and lignin derivatives as suberin and paraffinic structures are frequently detected in OREC due to the hydrophobicity of these compounds and the resistance to be oxidated (Alexis et al. 2012; Knicker et al. 2007; Rivas et al. 2012). Moreover, we observed higher values of aliphatic C after fire-induced charred plant deposition (3 days post-fire) in agreement with several studies that detected increases of hydrophobic organic compounds in the soil surface after fire (Almendros et al. 1992; Fernández et al. 2004; Knicker et al. 2005).

Shrubland was the only plant community that increased OREC and OREN fractions from the year 6 to 12 post-fire coinciding with a drought period. This plant community showed the highest necromass inputs during drought (Martí-Roura et al. 2011). Thus, the drought-induced accretion of organic matter observed between year 6 and 12 post-fire could account for the accumulation of recalcitrant substances. The higher values of alkyl C after this drought period could indicate the accumulation of hydrophobic organic compounds in the soil due to death of roots and shoots rich in suberin and waxes.

Surprisingly, a high proportion of O-alkyl C—considered labile C compound—was found in all the samples. This may indicate that hydrophobic compounds may have encapsulated labile O-alkyl-C into their hydrophobic alkyl-C network. In this way, this labile hydrophilic C may have been out of the reach of the dichromate oxidation. Indeed, other authors have found a high proportion of O-alkyl C, even after using the dichromate oxidation method at higher temperature (60°C) (Knicker et al. 2007).

¹⁵N incorporation into the OREC fraction

The application of biochar in soils can reduce nutrient leaching as they increase nutrient sorption by cation exchange mechanisms (Glaser

et al. 2002; Lehmann et al. 2003; Major et al. 2010). Yao et al. (2012) observed that most of the biochars they studied had the ability to sorb NH_4^+ and reduce leaching in biochar-amended soils. N- NH_4^+ can be incorporated to aromatic nuclei through heterocyclic rings in SOM rendering this N less available for plants and microorganisms (Burge and Broadbent 1961). In agreement with these findings, we observed that a small part of the $^{15}\text{N-NH}_4^+$ applied in burned soils was quickly incorporated in the most refractory fraction in all vegetations and correlated positively with OREC and OREN pool immediately after fire.

Pyrogenic materials and specially the aromatic structures (black C) have a long-term stability and are generally considered highly resistant against decomposition. Incubation studies carried out after applying charred materials in soils either under laboratory conditions (Baldock and Smernik 2002; Shindo 1991) or field observations (Major et al. 2010) confirmed the low respiration and mineralization losses of black C and the high mean residence time (MRT) of this compound calculated to thousands of years (e.g. at mean annual temperature of 10°C, MRT of 3264 years (Major et al. 2010); 4035 years (Liang et al. 2008)). Conversely, some recent studies found that aromatic structures could be less stable than it would be expected compared with some highly stable aliphatic structures like cutins or suberins (Alexis et al. 2012, Derenne and Largeau 2001). Although less is known about fire-induced recalcitrant N—estimated as OREN in our study—and its persistence and turnover rates, it is widely assumed the contribution of this N fraction to the refractory N pool. Conversely, our results showed that OREN derived from a fire was not irreversibly retained in the fraction, as $^{15}\text{N-NH}_4^+$ in the OREN fraction was remobilised in the following years as shown in mixed shrub-grassland (Fig. 2). This remobilisation represented a 10.6 % of the ^{15}N -tracer retained in the fraction three days after the fire. The remobilisation and deposition of ^{15}N -rich plant necromass during the drought period occurred between year 6 and 12 post-fire in plant communities without legumes could have hidden this effect.

Conclusions

The most refractory organic matter (OREC and OREN) increased after fires, mainly due to organic matter deposition after fire. This effect was independent from the intensity of fire and was mainly attributed to aliphatic structures. Fire slightly affected aromatic composition of organic matter.

Low biomass plant communities (grassland and mixed shrub-grassland) showed no changes in the OREC and OREN over 12 years. On the other hand, in communities with higher biomass (shrubland) the accrual of organic matter on the top soil during drought periods accounted for the accumulation of refractory compounds mostly formed by aliphatic structures.

Part of the mineral N released after fires was quickly incorporated into the most refractory N structures. However, the over-time variation of this newly incorporated N—that was observed in the mixed shrub grassland—questioned the stability of those highly resistant N compounds in soil.

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Discussió general



1 DISCUSSIÓ GENERAL

1.1 Resposta al foc segons el tipus de vegetació

En un incendi forestal, tant les característiques del foc com les entrades de matèria orgànica al sòl estaran condicionades pel tipus de vegetació existent en un indret determinat. Així, comunitats vegetals amb poca biomassa, com és el cas del nostre estudi, seran escenari d'incendis de moderada a baixa intensitat i és esperable que poc després del foc no hi hagi grans entrades de matèria orgànica al sòl (Almendros et al. 1988; Almendros et al. 1990; Knicker et al. 2005). Els canvis més importants van tenir lloc a les vegetacions amb un estrat arbustiu definit —brolla de romaní (shrubland) i prat emmatat d'argelaga (mixed shrub-grassland)— degut, molt probablement, al major aport de necromassa vegetal al sòl en comparació de la que va poder aportar els prats de llistó (grassland). Pel que fa a l'estoc de N total, la variació deguda al foc va ser petita i més difícil de detectar que els canvis en matèria orgànica (Article 2). De fet, les pèrdues de N per volatilització poden tenir lloc a temperatures relativament més baixes (200°C) que les de C. De tota manera aquestes pèrdues es poden veure compensades per una entrada de N provinent de material vegetal parcialment cremat. D'altra banda, es va observar que els valors de N mineral després del foc van ser de 3 a 6 vegades més grans a les parcel·les cremades que a les parcel·les control (no cremades), probablement degut a la deposició de cendres i a l'augment de mineralització de N provinent de l'escalfament del sòl (Raison 1979; Rapp 1990). El marcatge del N mineral ($^{15}\text{N-NH}_4^+$) ens va permetre fer el seguiment de la incorporació d'aquest N al sòl i observar l'evolució d'aquest dins l'ecosistema al llarg del temps. El N mineral alliberat després del foc es va incorporar ràpidament al sòl, principalment a la capa més superficial (0-2.5 cm) (Fig. 4.1), però una gran part d'aquest també, es va perdre durant els primers mesos després del foc.

Les diferents estratègies de regeneració de la vegetació després de l'incendi també van contribuir en gran part amb els canvis en el N mineral alliberat després del foc. Els baixos valors de ^{15}N recuperat al sòl 9 mesos després dels focs en els prats emmatats de llistó i argelaga (Fig. 4.1), no van

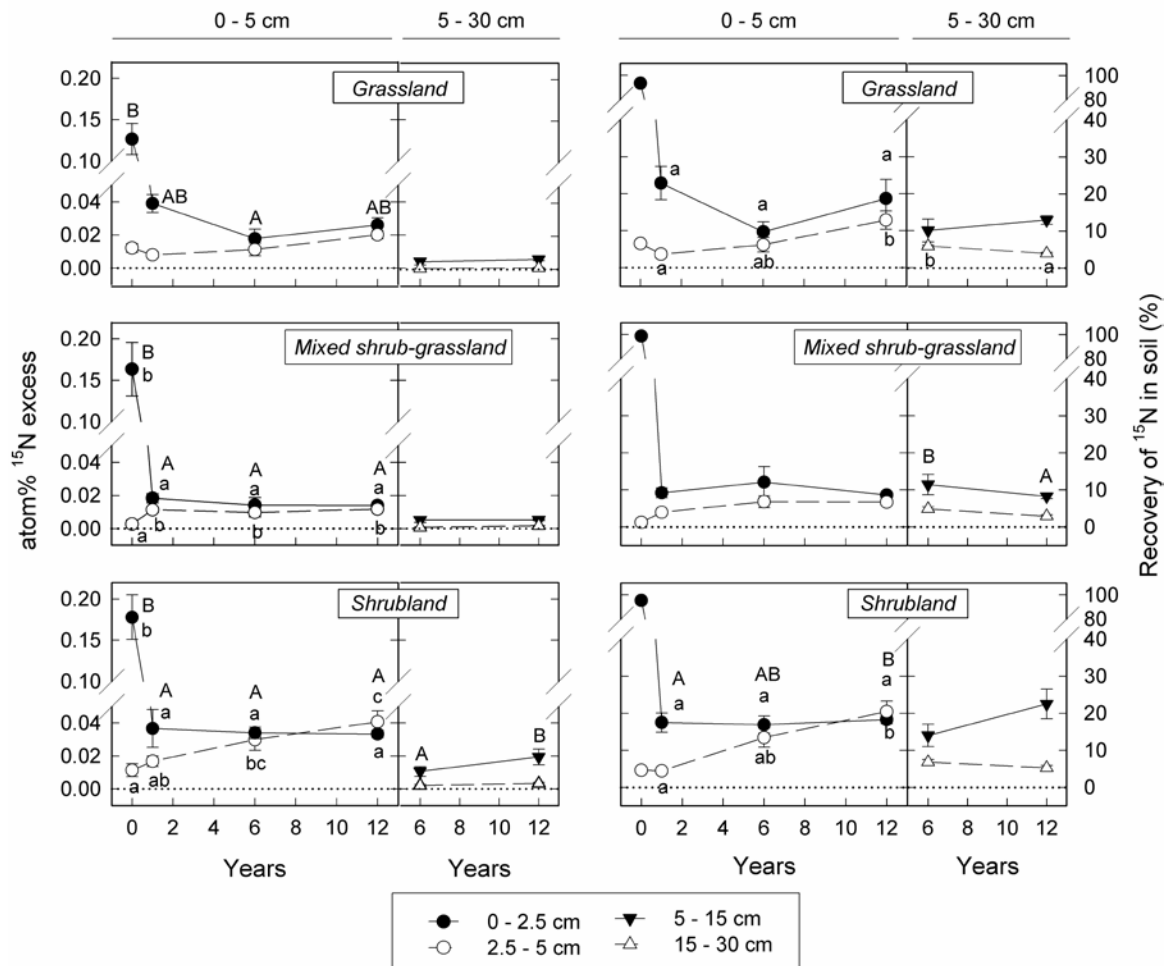


Fig 4.1 Enriquetament de ¹⁵N (Atm% ¹⁵N excess) i recuperació de ¹⁵N (%) a diferents profunditats per cadascuna de les comunitats vegetals (prat de llistó, matollar emmatat de llistó i argelaga i brolla de romani) 3 dies, 9 mesos, 6 anys i 12 anys després del foc. Les lletres majúscules indiquen diferències significatives al llarg del temps, integrant les dues profunditats mostrades a cada gràfic. Les lletres minúscules indiquen diferències significatives en cadascuna de les profunditats al llarg del temps. **Article 2, figura 2.**

poder ser explicats per un efecte del recobriment, ja que aquest va ser similar al dels prats de llistó. En canvi, la diferent qualitat de les restes vegetals i els residus cremats a les diferents vegetacions van poder tenir a veure amb els canvis de ¹⁵N recuperat 9 mesos després del foc. L'argelaga, com a planta fixadora de N atmosfèric, té un enriquetament extra de N als seus teixits, de manera que, deposicions de material vegetal enriquet en N com el provinent d'aquest tipus de plantes poden promoure processos de mineralització de N i nitrificació (Kuzyakov et al. 2009; Madritch i Cardinale 2007) i pèrdues de N per desnitrificació (Baggs et al. 2000; Huang et al. 2004; Millar et al. 2004; Zhong et al. 2011). Malgrat Romanyà et al. (2001), no van observar augments en la nitrificació de les parcel·les de llistó amb argelaga, grans quantitats de N mineral

(més del doble del dipositat per altres vegetacions) van ser aportats després de l'incendi amb el conseqüent risc de rentat durant els primers mesos després del foc.

1.2 Efectes del foc a llarg termini: Interacció amb la variabilitat climàtica

En un estudi de camp, les condicions climàtiques condicionen la resposta de l'ecosistema a una pertorbació i en poden facilitar la ràpida recuperació. Així doncs, l'evolució del sòl i la recuperació de les plantes després d'un incendi forestal estaran totalment condicionats a aquestes condicions climàtiques i ambientals, tan a curt com a llarg termini. En el nostre estudi ens vam trobar amb diferents episodis climàtics que van marcar el reciclatge de nutrients i l'acumulació de matèria orgànica al sòl.

En primer lloc un període de pluges molt intenses va marcar els primers mesos després del foc. L'augment de la reserva de N mineral que s'observa

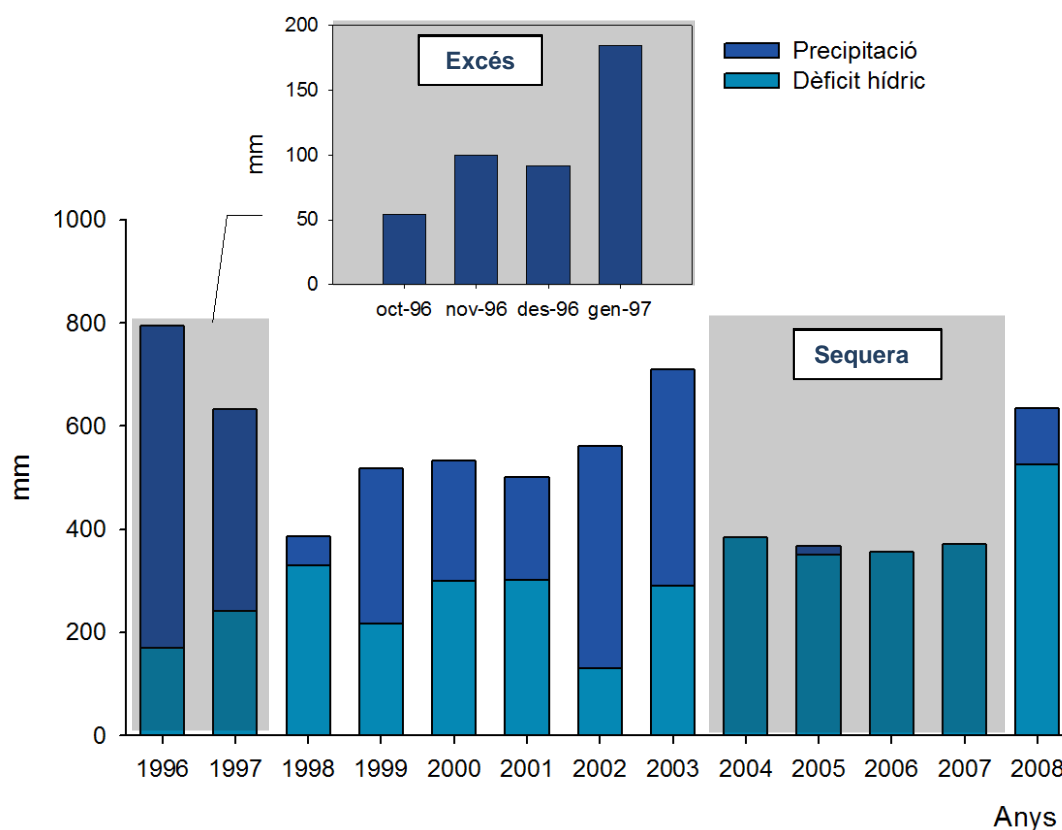


Fig. 4.2 Precipitació i dèficit hídric anual durant els anys d'estudi.

després dels focs, degut a la manca transitòria de vegetació pot no ser absorbit per les plantes i, per tant, pot perdre's ràpidament per rentat si les condicions meteorològiques ho propicien. En el nostre cas les pluges van ser molt abundants en els primers moments, arribant a precipitacions de fins a 376 mm durant els 3 primers mesos després dels focs (Fig. 4.2). Paral·lelament a les pluges, l'augment dels processos de nitrificació (en el cas de les brolles de romaní i els prats de llistó) o bé els nivells alts de per sí del N-NO_3^- després dels focs (en el cas dels prats emmatats de llistó i argelaga) (Romanyà et al. 2001) possiblement van promoure pèrdues per rentat de N-NO_3^- del sistema. Aquestes pèrdues no només es van veure traduïdes en el descens de la recuperació de ^{15}N durant els primers 9 mesos després del foc (Fig. 4.1), si no que també es va veure reflectit en una baixada del N total (Fig. 4.3).

Durant el període 2004-2007 (8-11 anys després dels incendis) la manca d'aigua va ser un dels factors més limitants per la supervivència de l'ecosistema (Fig. 4.2). Tot i la bona adaptació de les comunitats vegetals estudiades a certes condicions climàtiques extremes, la capacitat de supervivència de les plantes es va veure limitada després d'un període de sequera extrem amb una durada de 4 anys consecutius. Els períodes prolongats de sequera no només poden contribuir en l'aport de matèria orgànica al sòl, si no que també poden fer disminuir la biomassa microbiana i l'activitat enzimàtica (Bottner 1985; Zornoza et al. 2006), promovent l'acumulació d'aquesta matèria orgànica al sòl. Aquest període climàtic extrem es va veure traduït amb un augment de C orgànic al sòl, no només a nivell superficial, si no també en profunditat sobretot en les comunitats emmatades (Fig. 4.3). El sistema radicular de les plantes de vegetació mediterrània està molt ben desenvolupat i pot arribar a representar el 50% de la biomassa total (Caturla et al. 2000; Sánchez-Blanco et al. 2004). Conseqüentment, l'aport de material vegetal en cas de que aquestes es morin pot ser molt important també a nivell subterrani. La resposta a la sequera del C orgànic al sòl es va fer evident però no va ser tan clar amb el cas del N (Fig. 4.3). Els resultats van suggerir que la mort de part de la vegetació induïda per la sequera va provocar la incorporació de material vegetal i arrels grolleres al sòl rics en cel·lulosa, hemicel·lulosa o lignina i, per tant, amb una relació C:N alta donant lloc a una contribució més aviat pobra del N total a l'ecosistema edàfic. A

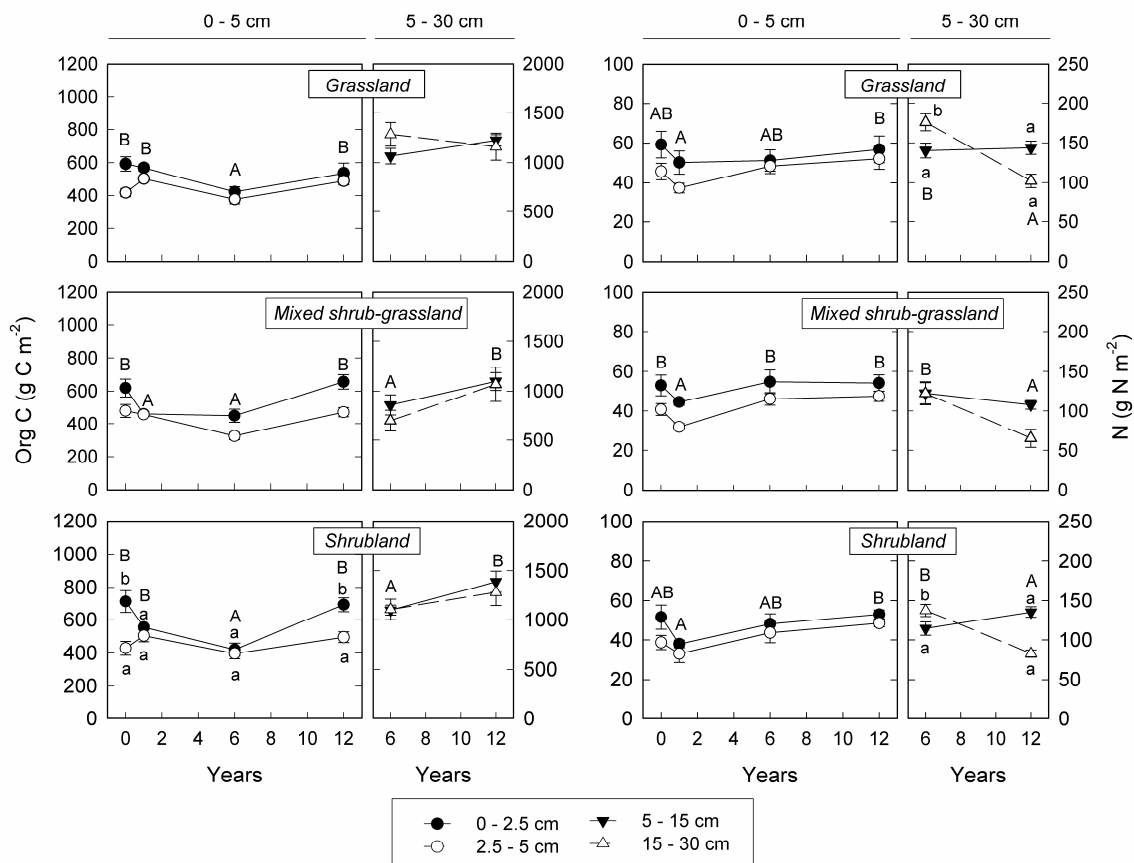


Fig. 4.3 C orgànic i N total al sòl a diferents profunditats i per cada comunitat vegetal (prats de llistó, prats emmatats de llistó i argelaga, brolla de romaní) 3 dies, 9 mesos, 6 anys i 12 anys després del foc. Les lletres majúscules indiquen diferències significatives al llarg del temps, integrant les dues profunditats mostrades a cada gràfic. Les lletres minúscules indiquen diferències significatives en cadascuna de les profunditats al llarg del temps. **Article 2, figura 1.**

més profunditat (15-30 cm) però es van observar pèrdues importants de N després d'aquest període de sequera, que van poder ser promoguts per un augment de la mineralització del N degut a l'entrada de nou material orgànic.

Les diferències en l'estoc de C orgànic entre les parcel·les cremades i les control van desaparèixer 6 anys després del foc, de manera que l'evolució posterior d'aquestes va ser en paral·lel. Així, durant el període de sequera, el que va marcar clarament l'evolució del N i el C orgànic del sòl van ser les diferents vegetacions i l'edat i mida de les plantes, especialment de les arbustives. També va poder ser rellevant la resistència de cada espècie a la manca d'aigua. Per exemple a partir de la corologia del romaní i de l'argelaga i de les propietats malacolífil·les del romaní podem inferir que aquesta espècie serà més resistent a la sequera que l'argelaga. D'altra banda, les diferències

observades en el C orgànic en profunditat fa pensar que els diferents patrons radiculars hi van tenir un paper important. Mentre que les brolles de romaní i els prats emmatats de llistó i argelaga van augmentar el C orgànic fins a 30 cm de profunditat, els prats purs de llistó amb una biomassa aèria molt menor i una biomassa subterrània superficial, no van mostrar grans canvis. Es va observar que durant aquesta època l'augment de C orgànic al sòl va ser major a les parcel·les control, amb una població vegetal més envellida i potencialment més sensible a l'estrès hídric, que a les parcel·les cremades, amb plantes més joves. A més, l'estratègia regenerativa de les plantes també va marcar l'evolució de les comunitats vegetals a llarg termini, tal com van mostrar les simulacions realitzades amb el model Roth C (article 1). Malgrat no saber les entrades reals de matèria orgànica durant el període de sequera, es va calcular a partir d'ajustos amb el model Roth C que les entrades de C necessàries per poder d'obtenir els valors de C que nosaltres vam trobar al sòl durant la sequera haurien d'haver estat molt grans. Aquests aportes de necromassa van ser relativament més destacats a les parcel·les amb arbustos rebrotadors (prats emmatats de llistó i argelaga), possiblement com a conseqüència de la seva menor tolerància a l'estrès hídric i la menor eficiència en l'ús de l'aigua en comparació amb altres espècies germinadores com ara el romaní (Paula i Pausas 2006).

Malgrat no es van observar canvis en el N total del sòl, sí que es va observar canvis en el ^{15}N del sòl, provinent de l'assimilació del N mineral alliberat després del foc durant el restabliment de la vegetació després de l'incendi. El ^{15}N recuperat va augmentar al sòl fruit del període de sequera mitjançant la deposició de material vegetal amb un alt contingut de ^{15}N en els seus teixits. Aquests augments van afectar principalment els primers 15 cm de sòl, però només a les comunitats vegetals lliures de lleguminoses (brolles de romaní i prats de llistó). En el cas de els prats de llistó amb argelaga, no s'observa cap canvi en el ^{15}N del sòl. Tot i que, la contribució en el marcatge de l'argelaga va ser pràcticament nul degut l'empobriment en ^{15}N dels seus teixits, la biomassa i marcatge de ^{15}N del llistó va ser similar que en els prats de llistó sense argelaga, de manera que es podia esperar un augment durant la sequera encara que més moderat. Això no va ser així, de manera que un augment en la descomposició de

les restes vegetals com a conseqüència d'un aport extra de N al sòl, facilitat per les lleguminoses, podria ser el causant de la manca de resposta del ^{15}N davant la sequera en aquestes comunitats.

1.3 Mecanismes de retenció de N al sòl

Bona part del N alliberat després del foc, va quedar retingut al sòl tal com es va observar a l'article 2. La ràpida retenció d'aquest N al sòl va permetre que una part del N quedés distribuït en les diferents fraccions granulomètriques del sòl, impedit-ne la pèrdua neta de l'ecosistema i, permetent-ne, en part, la seva assimilació posterior per part de les plantes que van tornar a créixer després del foc. Els mecanismes de retenció d'aquest N al sòl poden ser diversos i dependran de les propietats intrínseques del sòl, de la quantitat i qualitat de la matèria orgànica del sòl i dels processos biològics associats. Així doncs, l'estabilització del N al sòl va poder tenir lloc mitjançant dos mecanismes principals: (1) protecció del N a la matriu del sòl mitjançant processos físics (o fisicoquímics) (Article 3); (2) incorporació del N a la matèria orgànica recalcitrant per processos químics i bioquímics (Articles 3 i 4).

La incorporació de matèria orgànica a partícules fines permet protegir-la físicament de la degradació ja sigui per la dificultat que tenen els organismes d'accedir a aquesta per descomposar-la o bé per la formació d'enllaços organo-minerals molt estables (Mayer 1994; Skjemstad et al. 1993; Skjemstad et al. 2002). Així, el fraccionament granulomètric de la matèria orgànica ens va permetre observar la distribució de la MOS entre fraccions granulomètriques amb diferent grau de protecció física i la incorporació del N alliberat després del foc dins de cada fracció. Després del foc un dels efectes observats va ser la redistribució del N en diferents fraccions de sòl. A les vegetacions amb arbustos on la biomassa era major, el foc va tenir un efecte esmicolador de les restes vegetals que va ser traduït en el nostre estudi amb l'aparició de valors de N total més elevats a fraccions de mida intermèdia (sorra fina i llim groller) just després del foc (Fig. 4.4).

En el nostre estudi, tot i els augments detectats en el N a les fraccions de sorra fina i llim groller, els canvis en la recalcitrància després del foc van ser nuls o fins

i tot van disminuir en els prats de llistó ($p < 0.1$) i llistó amb argelaga ($p < 0.05$). Pel que fa al N mineral marcat va ser incorporat ràpidament després del foc quedant retingut principalment a les fraccions més fines. L'alta solubilitat del marcatge ($^{15}\text{N-NH}_4\text{Cl}$) va fer que quedés incorporat ràpidament a les fraccions més protegides (aprox. 29% de la recuperació de ^{15}N). Les argiles no només poden afectar l'estabilització del N orgànic mitjançant la protecció d'agregats, si no que el N també hi pot ser estabilitzat mitjançant la fixació de l' NH_4^+ a l'estructura cristal·lina de les argiles. Nannipieri et al. (1999) va observar que sòls rics en argiles fixadores d'amoni podien retenir fins un 30 % de la urea marcada amb ^{15}N aplicada en conreus. Una part important del N mineral va ser també incorporat a la fracció grollera (aprox. 10% de la recuperació de ^{15}N) degut a la possible contribució de processos microbians incorporant aquest N a la matèria orgànica d'aquesta fracció. A més, aquesta nova entrada de compostos rics en N mineral, tant a les fraccions grolleres com fines, va promoure també una disminució de la recalitrància d'aquestes fraccions. El comportament de les diferents comunitats vegetals, també va tenir un paper molt important en la incorporació d'aquest N mineral tant en els moments inicials, com amb la seva detecció al llarg del temps. Així, es va observar que mentre que les comunitats de rebrotadores (prat de llistó i prat emmatat de llistó i argelaga) el ^{15}N incorporat a la part recalitrant de les diferents fraccions es va mantenir després de l'incendi, a les comunitats de germinadores (brolla de romaní) el ^{15}N de la fracció recalitrant va disminuir després dels focs. En aquesta comunitat de mates germinadores hi ha una manca d'aports llenyosos durant els primers anys després del foc. Aquest fet suggereix, que a la brolla de romaní l'aport de material vegetal durant els primers anys post incendi va ser pobre en materials lignificats i/o suberificats que poden aportar recalitrància al sistema. L'estabilització del N en les diferents partícules de sòl també es va veure afectada pel període de sequera extrema que van patir les parcel·les sobretot on la biomassa va ser major. Les parcel·les amb vegetació arbustiva van tenir un augment del N total a les fraccions més grolleres com a resultat de la incorporació de material vegetal groller durant aquesta època. Paral·lelament al que es va observar en el total del sòl (Fig. 4.1), els augments de ^{15}N durant aquest període només van tenir lloc a les comunitats vegetals lliures de lleguminoses. Concretament, les fraccions més grolleres i més fines són les que van tenir major incorporació de ^{15}N al sòl després de la sequera

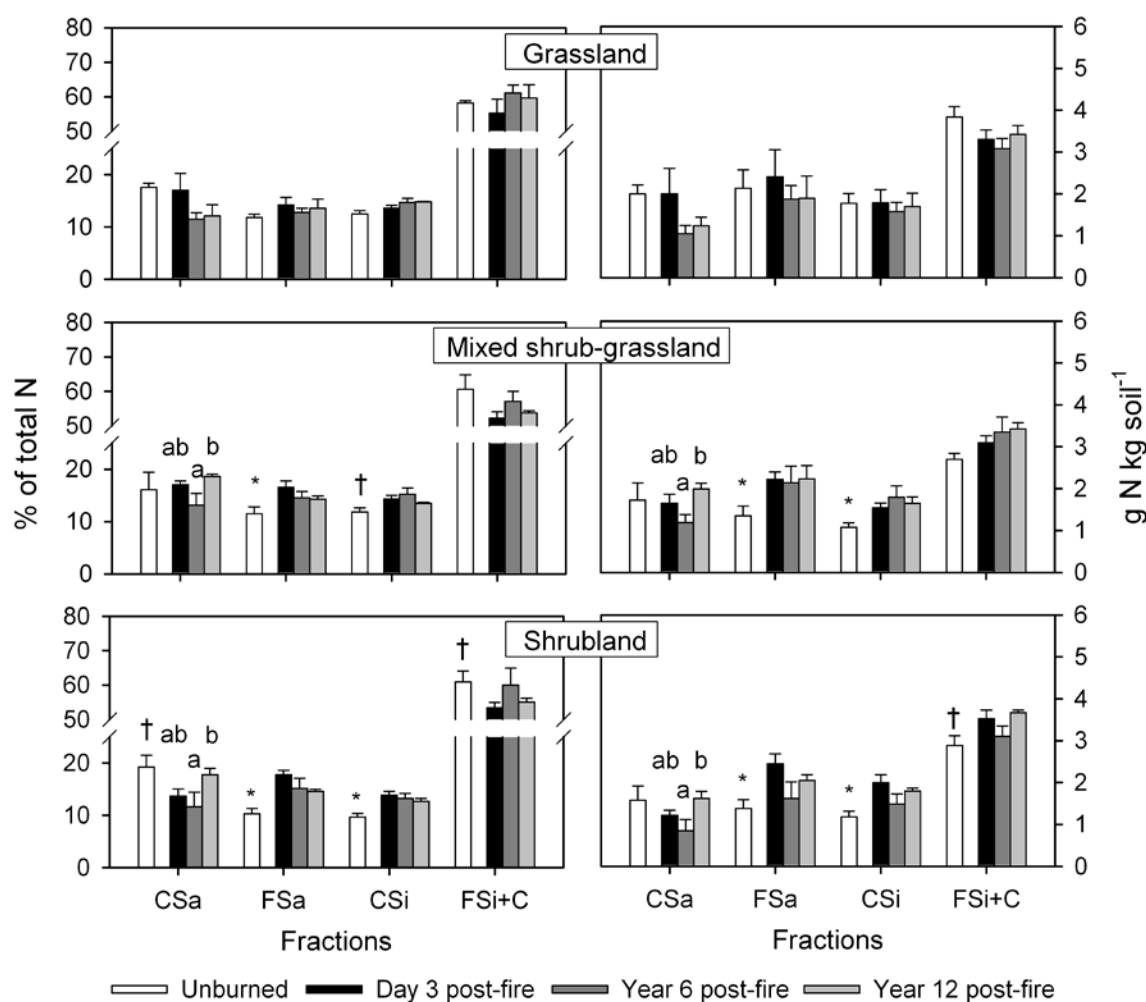


Fig. 4.4 Distribució del N total (%de N total) en les diferents fraccions granulomètriques (sorra grollera (CSa), sorra fina (FSa), llim groller (FSi) i llim fi+argila (FSi+C)) de sòls cremats i no cremats (3 dies, 6 anys i 12 anys després del foc) a les diferents vegetacions (prats de llistó, prats emmatats de llistó i argelaga i brolles de romaní). Les diferències entre les parcel·les cremades i no cremades 3 dies després del foc són marcades amb † $p < 0.1$ i * $p < 0.05$. En un mateix gràfic, les diferències entre diferents temps estan indicades amb diferent lletra a $p < 0.05$. **Article 3, figura 1**

però, en canvi, totes les fraccions van tenir un augment en la recalcitrància després d'aquest període, incloses les fraccions fines més protegides.

Una petita part del N marcat es va recuperar a la fracció més refractària de la matèria orgànica (article 4). Aquesta fracció, molt estable, es correspondria amb la matèria orgànica inerta i, tradicionalment, s'ha associat a grups aromàtics policondensats derivats en bona part de processos pirogènics resultants del foc i de l'escalfament del sòl. En el nostre estudi la matèria orgànica més refractària es va obtenir mitjançant l'anàlisi de COER i NOER (en anglès OREC i OREN) —

Carboni orgànic elemental recalcitrant i N orgànic elemental recalcitrant, respectivament—. Es va observar que els canvis en el COER i el NOER després del foc van anar íntimament relacionats amb els canvis en el C orgànic i en el N total del sòl i només els prats de llistó, que van patir l'incendi de menys intensitat, van tenir una acumulació neta de COER i NOER després del foc. Els nostres resultats coincideixen amb altres estudis realitzats on es va observar que els focs forestals no necessàriament han d'anar associats amb canvis en la quantitat de la matèria orgànica refractària (Rivas et al. 2012; Rovira et al. 2009). Només una petita fracció del N mineral alliberat després del foc va unir-se als residus més refractaris de la matèria orgànica (2-3% del ^{15}N recuperat) però d'una forma no irreversible. El $^{15}\text{N-NH}_4^+$ incorporat en el NOER va ser mobilitzat al cap dels anys en vegetacions sense lleguminoses. En el cas dels prats emmatats de llistó i argelaga, les deposicions de material empobrit en ^{15}N podrien haver emmascarat aquesta remobilització. Per tal d'estudiar més en detall la composició de la matèria refractària es van analitzar els residus dels sòls de les brolles de romaní per $^{13}\text{C-NMR}$. Els resultats obtinguts van mostrar una major abundància de compostos de caràcter hidròfob (C alquil) en comparació amb els compostos aromàtics, que només van representar un 2 % del C orgànic. A més, el canvi més destacat després del foc va ser precisament amb l'augment de l'abundància de compostos hidròfobs i és que, freqüentment s'han detectat augments en la hidrofobicitat en la superfície dels sòls després d'incendis forestals degut a la deposició de derivats de lignines i lípids com suberines i parafines, molt resistents a l'oxidació (Almendros et al. 1992; Fernández et al. 2004; Knicker et al. 2005). Certes estructures alifàtiques estables com cutines i suberines han demostrat tenir una gran estabilitat al sòl, fins i tot major que els compostos aromàtics (Alexis et al. 2012; Derenne i Largeau 2001). A més la dinàmica d'aquests productes no té perquè anar lligada al foc ni a l'escalfament del sòl a temperatures no fisiològiques.



Conclusions



Conclusions

1. La baixa intensitat dels focs forestals en brolles i prats secs mediterranis va provocar que els canvis quantitius en el C orgànic i en el N total del sòl no fossin gaire destacats després del foc i que la recuperació davant d'aquests canvis fos molt ràpida.
2. Un període prolongat de sequera va provocar l'augment d'aports de matèria orgànica al sòl provinents de l'augment de la necromassa a l'ecosistema. Aquest fet tingué lloc tant a les parcel·les cremades com a les control i va augmentar els continguts de C orgànic i, en menor grau de N del sòl, principalment a les comunitats vegetals amb espècies arbustives on la biomassa era major que als prats. La magnitud dels aports de matèria orgànica al sòl durant el període de sequera va ser determinada per la l'edat i la mida dels arbustos i la sensibilitat de les espècies vegetals a les condicions de sequera.
3. Els estocs de C orgànic i N total al sòl a llarg termini van demostrar ser més sensibles al canvi davant la variabilitat climàtica que a un incendi forestal aïllat.
4. Una bona part del marcatge amb ^{15}N aplicat pel seguiment del N mineral alliberat just després del foc, al cap de 12 anys, encara estava retingut al sòl a les comunitats vegetals sense plantes lleguminoses. En canvi, la presència de lleguminoses va afavorir les pèrdues de ^{15}N des del primer any després del foc i també durant el període de sequera.
5. Després del foc es va observar un augment de N a les fraccions granulomètriques intermèdies com a conseqüència de l'entrada de material cremat particulat. Aquest efecte es va mantenir al llarg de 12 anys després del foc.
6. El N mineral alliberat després del foc es va incorporar principalment a la fracció més grollera ($>200\ \mu\text{m}$) i a la fracció més fina del sòl ($<20\ \mu\text{m}$). El N mineral incorporat a les fraccions fines va associar-se a la matèria orgànica més làbil tot i la protecció física que presenten aquestes partícules.
7. La matèria orgànica més refractària (COER i NOER) va augmentar després dels focs, principalment degut a un augment en la deposició de matèria orgànica

no cremada. Aquest efecte va ser independent de la intensitat dels focs i va ser principalment atribuït a la presència d'estructures alifàtiques hidrofòbiques i no pas d'aromàtiques, que van ser poc importants.

8. A les comunitats vegetals amb més biomassa, cas de les brolles de romaní, la sequera va promoure també l'acumulació de matèria orgànica refractària amb dominància d'estructures alifàtiques hidrofòbiques.

9. Part del N mineral alliberat després del foc va ser ràpidament incorporat a les estructures més refractàries de la matèria orgànica del sòl. La variació al llarg del temps d'aquest N novament incorporat permet qüestionar la gran permanència al sòl d'aquest tipus de compostos orgànics.



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Annex

A study on '*Soil organic matter stabilisation in acid and alkaline soils of the Mediterranean area across different land uses*' was performed under the supervision of Dr. Frank Hagedorn at the department of Forest soils and Biogeochemistry at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) (Birmensdorf, Switzerland). This study was carried out in 2 stays of 3 months each one (September-December 2011 and January-April 2012)

Brief background and rationale

Microbial communities are the main decomposers of soil organic matter. However, so far it is not clear whether belowground SOM (Soil organic matter) turnover is mainly driven by the structure of the soil microbial communities or by the availability of soil organic matter sources (Blagodatsky et al. 2010; Strickland et al. 2009). The availability of SOM sources to microbes is primarily regulated by soil organic matter stabilisation processes (Lützow et al. 2006).

Soil fine particles (clay and fine silt) and carbonates are believed to minimize the turnover of SOM and soil acidity to slow down soil organic matter decomposition. While the role of fine particles and their soil minerals in organic matter stabilization is widely accepted, the role of soil pH and associated chemical soil properties such as the presence of carbonates is less clear and the results are even contradictory (Amato and Ladd 1992; DeLaune et al. 1981). On the one hand, soil pH is involved in the turnover of SOM by the regulation of biomass, activity and composition of soil microbial community (Adams and Adams 1983; DeLaune et al. 1981; Nodar et al. 1992; Shah et al. 1990; Zelles et al. 1987). On the other hand, in calcareous soils, it is well known that carbonates form aggregates with a high stability and, consequently, carbonates are actively involved in the protection of belowground C (Boix-Fayos et al. 2001; Olk et al. 1995). This high protective capacity could account for the large SOC (Soil organic carbon) stocks found in calcareous forest soils of the Mediterranean temperate area (Romanyà and Rovira 2011), and may involve relevant changes in soil microbial communities. Moreover, the stabilization mechanisms of carbonates

may operate differently in soils depleted of organic matter compared to soils close to organic matter saturation (Bronick and Lal 2005).

While SOM stabilization had been extensively studied in the temperate zone (e.g. (Guggenberger et al. 1994) much less is known for the Mediterranean area (e.g. (Rovira et al. 2010). In the Mediterranean area, grassland soils show unexpectedly low values of SOC stocks (SOC less than 1.5% in the top 25 cm) that are in contrast with the very high levels observed in forest soils (SOC about 3 % in the top 25 cm) of the same climatic zone (Romanyà and Rovira 2011). The SOC stocks are especially high in alkaline forest soils. In contrast, organic matter depleted grassland and arable alkaline soils show similar or even lower values than acid soils of the same land use and climatic area (Romanyà and Rovira 2011). Although there is some data on soil microbial diversity in alkaline Mediterranean soils with contrasted organic matter levels (Zornoza et al. 2009) no much is known about the linkage of soil microbial communities to soil pH and/or carbonate content.

The aim of this work was to study SOC stabilization and microbial communities associated to this process in the Mediterranean soils, in particular the effects of carbonates on SOM in soils of different land use. We hypothesized that, in calcareous soils, carbonates have an important role in the physical and chemical protection of organic matter. The protective role of carbonates would be different in soils depleted with organic matter (arable and grassland) where protective carbonate coating could have an important role in the physical protection of soil organic matter than in soils rich in organic matter (forest soils) where high content of free organic matter would become stabilised by other processes such as cationic bridging with Ca^{2+} .

Material and methods

Study site and soil sampling

The study included soils of different land uses (agricultural, grassland and forest) with different level of organic matter. This was done in two Mediterranean soil scenarios: soils with and without carbonates. Our approach was to characterise the microbial communities associated to each soil. The experimental design consisted of a 2 factor model: land-use and presence/absence of carbonates. Based on a previous soil survey, we selected soils from 6 different areas close to Barcelona: 3 with carbonated soils and 3 non-carbonated soils. All areas were similar in climate (Mediterranean) and different in parent material. In each area, we sampled soils from 3 different land uses: agricultural (arable), grassland and forest; with similar texture and similar climatic, geological and, when it was possible, topographic conditions. From each soil and land-use type we took samples from the upper 10 cm of the mineral soil from three different sites. Hence, for this study, a total number of 18 samples were taken (2 soil types x 3 land-uses x 3 replicates). For PLFA analysis moist subsamples of soil were sieved to <4 mm and immediately kept frozen until their analysis. For physical and chemical analysis and soil respiration measurements soils were dried and sieved to <2mm.

Soil physical and chemical analysis

Prior to chemical analysis soil samples were oven-dried at 60°C and sieved <2 mm.

Standard analyses: Texture was obtained by the pipette method (Porta et al. 1986) after H₂O₂ treatment to eliminate the organic cementing agents. Soil pH in water with glass electrode, with a proportion soil/water of 1:2.5 (w/v). A subsample of each sample was finely ground in an agatha mortar to analyze carbonate and organic C content. Carbonate content was obtained by the Bertrand's calcimeter method (Porta et al. 1986). Organic carbon by dichromate oxidation (Moebius 1960) at 155°C, using an aluminum block digester adapted to a colorimetric method (Soon and Abboud 1991). Total N was analyzed with an automated elemental analyser-continuous flow isotope ratio mass spectrometer

(Euro-EA, Hekatech GmbH, Germany, interfaced with a Delta-V Advanced IRMS, Thermo GmbH, Germany).

Fractionation method: The soil samples were fractionated into different particle size: coarse sand (2000-200 μm), fine sand (200-50 μm), coarse silt (50-20 μm) and fine silt+clay (<20 μm) (Rovira et al. (2010) and also widely explained in Article 3). This part was carried out at University of Barcelona. We analyzed the organic C from each sample as we mentioned before. The second part, which we went to perform at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), was to analyse calcium, aluminium and iron by wave-length dispersive X-ray fluorescence spectrometry (WD-XRF, Axios, PANalytical).

Lipid extraction and PLFA analysis

We assessed the PLFA of each bulk soil sample to characterize the nature of microbial communities. Moist soil samples were sieved (<4mm) and kept frozen until their analysis, which took place at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL). We determined the PLFA abundance of soils, which are biomarkers for specific groups of microorganisms, following the procedures described by Frostegard et al. (2011). Microbial fatty acids were extracted from 1g with a fresh soil with a monophasic mixture (1:2:0.8 v/v/v) of chloroform, methanol and phosphate buffer (Bligh and Dyer 1959; Zelles 1997). For separating the organic and the water phase, accessory water and chloroform was added. The lipids were fractionated into neutral lipids, glycolipids and phospholipids on silicic acid columns, impregnated with ammonium acetate. The phospholipid fatty acid were methylated at 60°C for 2 h using trimethylchlorosilane and methanol (1:9 v/v) (Thiel et al. 2001). 19:0 was added as an internal standard prior to the GC/MS measurement. For PLFA detection, an Agilent GC-mass spectrometry (MS) system (HP 6890 N Plus gas chromatograph connected to a 5973 N MSD detector) with 50 m capillary column (Agilent 128-552 DB5-5MS) was used. The PLFAs were identified by comparing the retention times of the samples with those of a standard mixture (bacterial acid methyl esters, Supelco Inc.) and by inspection of mass spectra. Standard nomenclature was used to describe the fatty acids. First number refers to the number of C-atoms, followed by the number of double bonds and their position

(ω). The prefixes “i” and “a” refer to iso- and anteiso-branched fatty acids. The prefix “cy” indicates cyclopropane fatty acids. 18:2 ω 6,9 was used as a biomarker for fungi, i15:0, a15:0, i16:0, i17:0, a17:0 for Gram positive bacteria, 16:1 ω 5, 16:1 ω 7, cy17:0, 18:1 ω 7 and cy19:0 for Gram negative bacteria (Zelles 1997; Zelles 1999) and 10Me16:0 for actinomycetes (Federle and Megusar, 1986). PLFA concentrations were calculated as $\mu\text{mol PLFA per gram C}$ and mol%.

Soil respiration

To investigate microbial activity, we incubated bulk mineral soils (0-10 cm depth) and soil size fractions (coarse sand, fine sand, coarse silt and fine silt+clay) for 119 days at 20 °C measuring soil respiration.

Microcosm preparation: We mixed 2 g of oven-dried ground soil with 2 g of ashed quartz sand in order to homogenize the different samples and were added to 5ml plastic vials. The plastic vials were placed into 120 ml glass bottles and sealed with gas tight lids equipped with septa to allow quantification of the CO₂ concentration in the headspace (Figure 1). A pre-incubation was carried out in order to activate microbial community in the oven-dried samples. Soils were rewetted to 60% of water holding capacity (WHC) and inoculated with an inoculum prepared by a mixture of fresh soil and water 1:10 (g:ml). The wetted soils were pre-incubated during 1 week at 20 °C.



Figure 1. Microcosm with a vial with the soil sample placed into a glass bottle and sealed with gas tight lids equipped with septa

Soil respiration: Soil respiration was measured by quantifying CO₂ concentration in the headspace within each bottle by an LICOR-840 infra-red gas analyser (IRGA). Measurements were done at day 0, 1, 7, 14, 20, 35, 49, 63, 77, 91, 105 and 119 after the start of the incubation. For each measurement period an initial

Table 1. Initial physicochemical average values and total PLFA concentration in soils with and without carbonates and with different land use (field, abandoned field and forest). Land use differences are labelled with a different lower case letter and differences between soils with or without carbonates are labelled with a different capital letter, at $p < 0.05$. Values are means \pm SE (n=3)

	- carbonates			+ carbonates		
	CFi	AFi	Fo	CFi	AFi	Fo
Organic C (%)	1.17 \pm 0.31	1.26 \pm 0.22 A	1.92 \pm 0.08 A	1.24 \pm 0.41 a	1.93 \pm 0.09 aB	4.49 \pm 0.51 bB
Total N (%)	0.104 \pm 0.026	0.142 \pm 0.031	0.158 \pm 0.019 B	0.120 \pm 0.030 a	0.162 \pm 0.010 a	0.279 \pm 0.016 b A
Total PLFA ($\mu\text{mol gC}^{-1}$)	4.62 \pm 0.61 a	8.00 \pm 0.09 bA	4.89 \pm 0.22 aA	8.85 \pm 3.33	4.85 \pm 0.49 B	2.70 \pm 0.18 B
pH	6.96 \pm 0.40 A	6.69 \pm 0.29 A	6.14 \pm 0.17 A	8.31 \pm 0.12 B	8.22 \pm 0.04 B	8.08 \pm 0.06 B
Carbonates (%)	0.11 \pm 0.05 A	0.19 \pm 0.09 A	0.02 \pm 0.01 A	59.73 \pm 3.03 B	56.38 \pm 3.16 B	54.74 \pm 5.52 B
Texture						
Sand (%)	50.9 \pm 0.7 A	49.3 \pm 4.2 A	51.5 \pm 1.7 A	25.5 \pm 1.9 B	31.9 \pm 2.0 B	32.0 \pm 4.7 B
Silt (%)	32.6 \pm 1.4 A	35.3 \pm 2.6	34.4 \pm 1.5 A	47.1 \pm 1.6 B	42.7 \pm 1.3	44.6 \pm 2.7 B
Clay (%)	16.5 \pm 0.7 A	15.4 \pm 2.1 A	14.1 \pm 1.5	27.4 \pm 0.7 B	25.4 \pm 1.4 B	23.4 \pm 5.5

ANOVA summary

	Land use	Carbonates	Land use*carbonates
Organic C (%)	<0.001	0.001	0.005
Total N (%)	0.002	0.016	0.071
Total PLFA ($\mu\text{mol gC}^{-1}$)	0.001	0.077	0.009
pH	0.960	<0.001	0.419
Carbonates (%)	0.679	<0.001	0.690
Texture			
Sand (%)	0.477	<0.001	0.392
Silt (%)	0.912	<0.001	0.229
Clay (%)	0.478	<0.001	0.952

measurement of the CO₂ concentration in the headspace was taken immediately after sealing the bottles. The closed bottles were then left to incubate for a defined duration (1 day from 0 to 7 days and 2 days for the rest of the incubation) and then a second measurement was taken. After the second measurement all the bottles were opened to renovate the air in the bottles and partially closed during the incubation time to avoid excess of water evaporation from the samples. The CO₂ respired for the microbial community was calculated as the difference between the initial and final CO₂ concentrations obtained for each measurement.

Results and discussion

Soil microbial community: structure and activity

All soil physical and chemical characteristics described in table 1, showed significant differences between soils with carbonates and without carbonates, while land use factor only had effects on organic C and total N contents and total PLFA. Despite soil organic C content was higher in forests (only significant in carbonated soils) than in cultivated and abandoned fields, forests showed the lowest values of total PLFA per gram of C (Table 1). As expected, TOC and total N content increased from the cultivated field to the forest (cultivated field < abandoned field < forest).

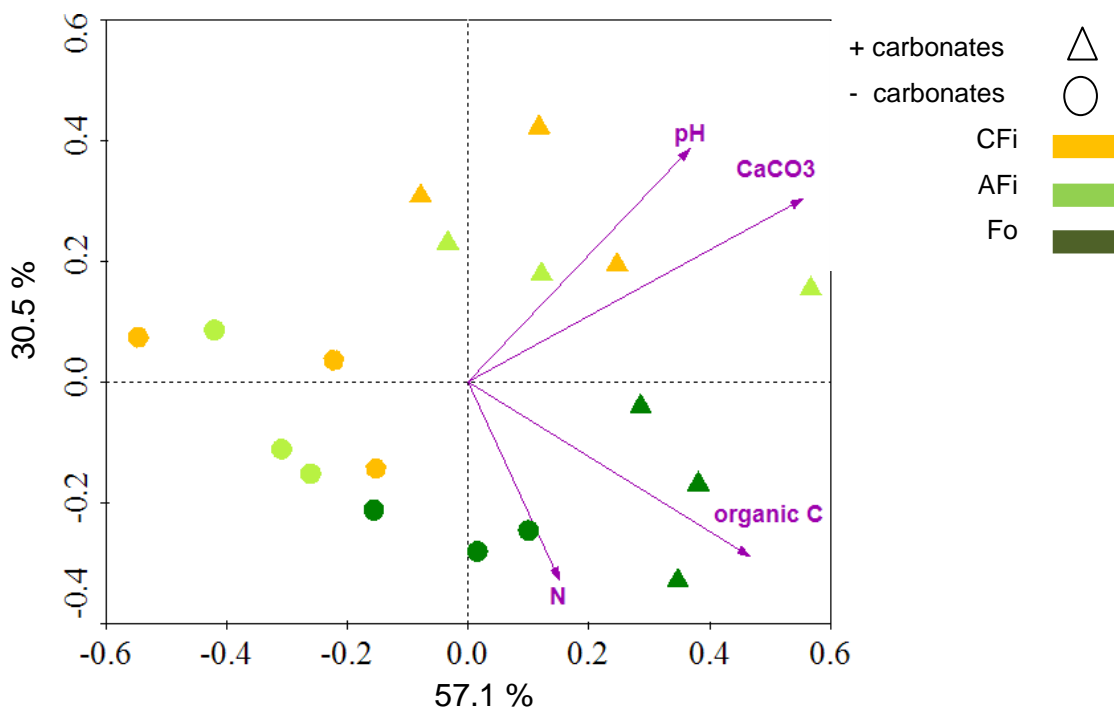


Figure 2. Canonical Correspondence Analysis (CCA) performed on the relative abundance of PLFA and soil characteristics (pH, CaCO₃ (%), organic C (%), Total N (%)) in soils with carbonates (triangles) and soils without carbonates (circles) and in all land uses: cultivated field (CFi) (orange symbols), abandoned field (AFi) (light green symbols) and forest (Fo) (dark green symbols).

Canonical Correspondence Analysis (CCA) carried out with all microbial PLFAs and soil characteristics, explained a 87.6 % of the variability in the first two axes. Carbonated and non carbonated soils were clearly separated by the first principal component (Fig. 2) and carbonated soils were positively correlated with pH and CaCO₃ content. On the other hand, forest soils were separated from

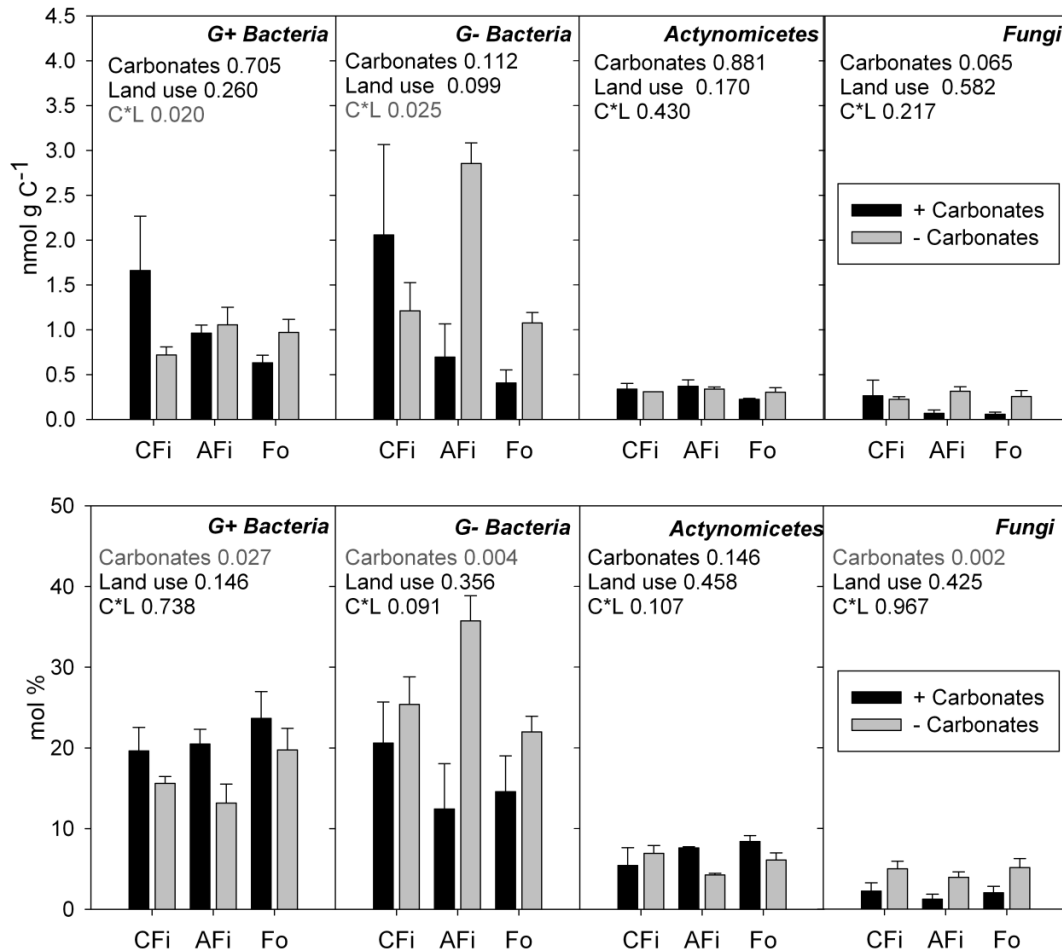


Figure 3. PLFA biomarkers representative for different microbial groups in all land uses (cultivated field (CFi), abandoned field (AFi) and forest (Fo)) and two types of soils (soils with carbonates and without carbonates). Error bars represent standard errors (n=3).

cultivated and abandoned fields by the second axis and, in this case, total N and organic C correlated positively with the microbial community variation in forest soils. Soil microbial community composition showed different patterns depending on the soil type and land use. In carbonated soils representative PLFA of gram positive and gram negative bacteria had higher values in fields than in forests (Fig. 3). However, in non carbonated soils the highest values for gram negative bacteria were in abandoned fields. Actynomicetes and fungi did not show significant differences in PLFA content in any case. However, the relative abundance of PLFA associated to fungi and gram positive biomarkers showed higher values in non carbonated soils than in carbonated soils. Other authors have also found high values in fungal PLFA marker in slightly acid soils (Rousk et al. 2009).

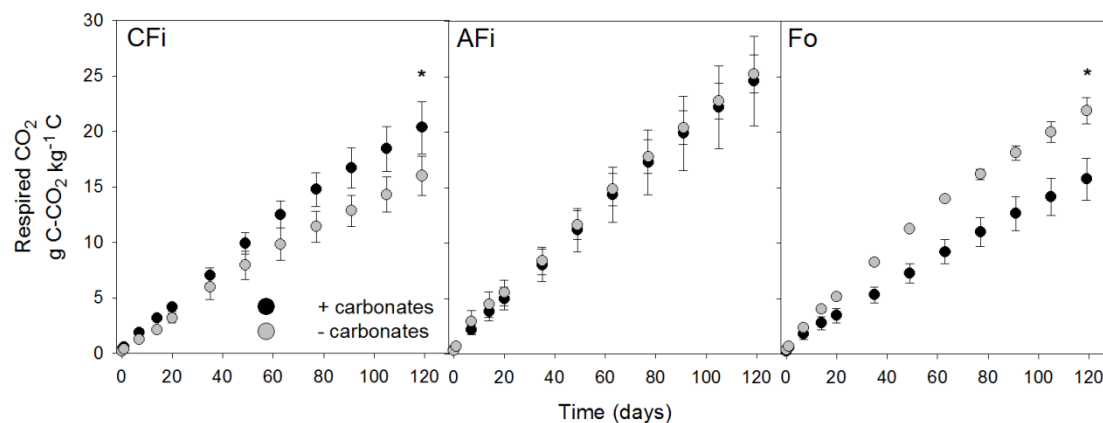


Figure 4. Cumulative carbon (C) mineralization of carbonated and non carbonated soils at three different land use: cultivate field (CFi), abandoned field (AFi) and forest (Fo). Means and standard errors of three plots.

Soil microbial respiration showed differences between carbonated and non carbonated soils depending on the land use (Fig. 4). In cultivated fields, C mineralization was higher in carbonated soils than in non carbonated soils. No differences were found between the two types of soils in abandoned fields and, in forests, lower C mineralization was found in carbonated soils compared with non carbonated soils. Fields from carbonated soils showed C mineralization rates relatively high considering their low soil organic C content, which was almost 4 times lower than forest soils with the similar chemical and physical characteristics (table 1). The high content of total PLFA and bacterial PLFA that were found in fields of carbonated soils coincided with large microbial activity. On the other hand, the high respiration rates observed in forest soils without carbonates coincided also with high PLFA results. However, in this case the differences concentrated on the relative abundance of fungal PLFA rather than that of bacteria.

Soil respiration and soil size fractions

Soil organic C content in all fractions showed the lowest values in cultivated fields and the highest in forest (CFi<AFi<Fo). Carbonates distributed differently depending on the land use and the size fraction. Coarse fractions and fields had the highest CaCO₃ content and fine fractions and forests had the lowest values.

Taula 2. Carbonates and organic C (%) contents in carbonated soils with different land uses: fields (Fi), abandoned fields (AFi) and forests (Fo); and different particle size fraction: coarse sand (CSa), fines and (FSa), coarse Silt (Csi) and fine silt+clay (FSi+C). Values are means \pm SE (n=3)

	CFi	AFi	Fo
CaCO₃ (%)			
Csa	83.2 \pm 5.2 a	75.9 \pm 8.2 a	66.4 \pm 6.6
Fsa	65.9 \pm 6.8 ab	55.8 \pm 5.7 b	55.4 \pm 6.8
Csi	51.4 \pm 7.2 b	46.1 \pm 5.2 b	39.5 \pm 6.7
FSi+C	53.7 \pm 5.8 b	48.2 \pm 2.0 b	41.6 \pm 7.0
Organic C (%)			
Csa	1.40 \pm 0.48	3.87 \pm 1.27	6.13 \pm 1.33
Fsa	1.28 \pm 0.41 A	1.82 \pm 0.28 A	4.72 \pm 0.70 B
Csi	1.10 \pm 0.43	2.27 \pm 0.66	2.88 \pm 1.11
FSi+C	1.39 \pm 0.52 A	2.12 \pm 0.10 A	4.07 \pm 0.60 B

ANOVA summary

	Land use	Fraction	Land use*Fraction
Carbonates (%)	0.027	<0.001	0.994
Organic C (%)	<0.001	0.060	0.481

In calcareous soils separate incubations of soil fractions showed lower respiration in fine fractions than in coarse fractions ($p=0.036$). In contrast, no changes were detected in non calcareous soils ($p=0.454$) (Fig. 5). In general, coarse fractions are considered more unprotected, to be more labile and readily available C source for microbes (Olk and Gregorich 2006), but surprisingly we only detected this pattern in calcareous soils. We also observed that only calcareous soils showed strong relationships between the abundance of Al, Fe and Ca and soil fraction respiration. These relationships mainly occurred in the coarse fractions and were positive for Ca and negative for Al and Fe. In contrast, in non calcareous soils separate fractions respiration was independent from these elements. Thus, it seems that the variability of Al and Fe at high pH ranges could influence soil fraction respiration while it did not at slightly acid pH (Fig. 6). We expected the carbonates in the coarse fractions to exert a protecting role but our data shows that soils with more carbonates and Ca had higher respiration rates. Somewhat surprisingly, these higher respiration rates per unit of organic C

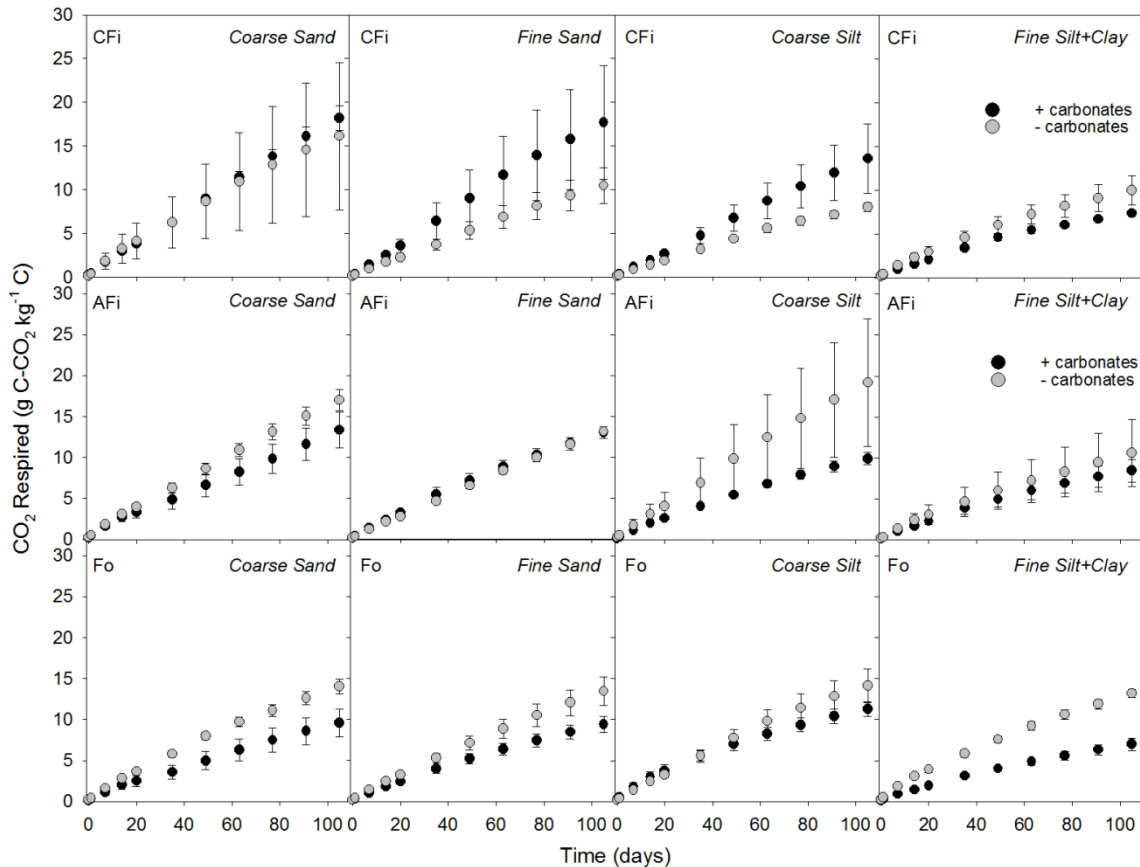


Figure 5. Cumulative carbon (C) mineralization of carbonated and non carbonated soils at three different land use (Cultivate field, Abandoned field and Forest) and for each soil fraction. Means and standard errors of three plots.

coincide with arable soils with very low organic matter levels. This suggests that in these soils carbonates do not protect from microbial attack the organic matter in coarse sand, fine sand and coarse silt fractions (see Fig. 5) while they may protect the organic matter in coarse fractions in soils holding high organic matter content such as forest soils and the organic matter in the fine silt+clay fraction irrespective of the land use. Thus, it appears that in low organic matter soils carbonates protection could have a more active role in fine particles than in coarse fractions.

In summary, soil carbonates can affect organic matter dynamics and microbial structure in different ways depending on the land use. While in forest soils carbonates protect soil organic matter throughout all particle size fractions, in organic matter depleted arable soils carbonate protection capacity concentrates in the finest fractions. In arable carbonated soils the enhanced

respiration rates in coarse fractions was not explained by the microbial community structure. In the context of organic matter depleted soils, the presence of carbonates in soil fine particles could have a more active role in protecting organic matter than in coarse fractions.

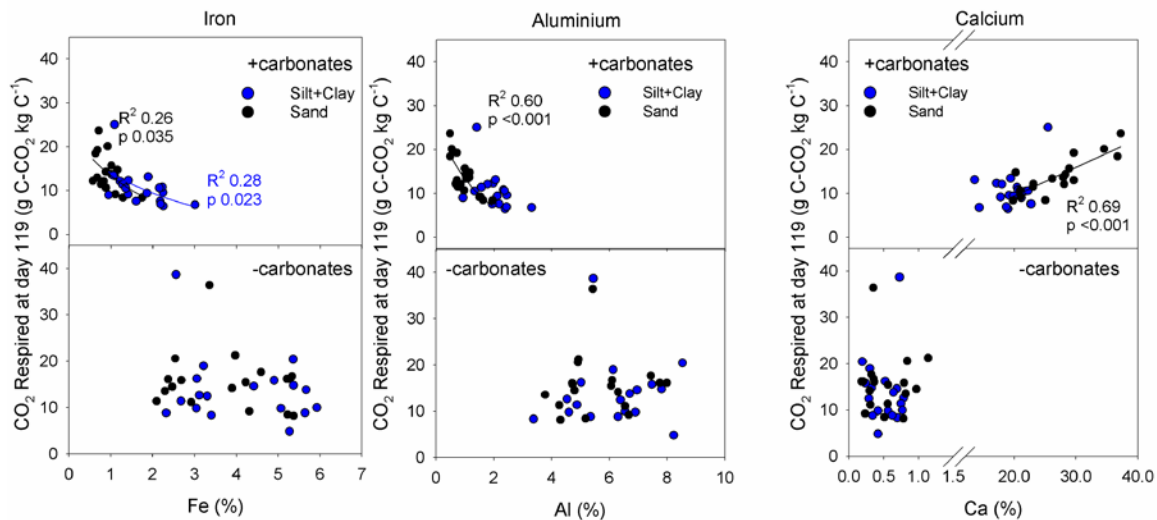


Figure 6. Relationship between accumulative CO₂ respired at day 119 of the incubation experiment and aluminium (Al), calcium (Ca) and iron (Fe). The lines are fitted to a exponential decay function (Al and Fe) and to a linear function (Ca).

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