TESIS DOCTORALES

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Estructura y dinámica de la materia orgánica del suelo en ecosistemas forestales templados: de lo particular a lo general

Nahia Gartzia Bengoetxea

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INGURUMEN, LURRALDE PLANGINTZA, NEKAZARITZA ETA ARRANTZA SAILA

DEPARTAMENTO DE MEDIO AMBIENTE, PLANIFICACIÓN TERRITORIAL, AGRICULTURA Y PESCA

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UNIVERSIDAD DEL PAÍS VASCO

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Eusko Jaurlaritzaren Argitalpen Zerbitzu Nagusia
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Etxekoentzat

Mendian lar artean aurkitzen da loretxo bat, aurrian umetxo bat loretxoari begira, loreak esan nahi dio, umetxo aska nazazu, jaio naiz libre izateko eta ez loturik egoteko. Umetxoak ikusirik lorea ezin bizirik, arantzak kendu nahi dizkio bizi berri bat eman, orduan izango bait du indarra eta kemena, orduan emango bait du ugari bere fruitua.

Benito Lertxundi

Esker ona

Eskerrak helmugara iritsi naizen! Mendi maratoi hau hasi nuenean helmuga non zegoen ere ez nekien eta berau aurkitzeko jende askok GPS moduan funtzionatu izan du.
Lehendabizi, Ander eta Inaziori bide luze honetan, hasieratik amaierara nire ondoan egotea eskertzen diet eta mendi malkartsuenetan motxilako pisua elkarbanatzen laguntzea. Eta noski, DAPAri motxila janariz betetzea ere eskertu behar diot.
Mendi martxa honetan, jende askok bidearen zati bat egiten lagundu izan dit eta egia esan, beraien laguntza behar-beharrezkoa izan da etapa bat bestearekin lotzeko. NEIKEReko lantalde osoari eskerrak zientziaren mundu zabala lurreratzen laguntzearren, eta batez ere Martari eskerrak lurzoru kimika pixkat ulergarriagoa egiten saiatzearren. Nafarroako Edafologia saileko Marijo, Iñigo, Paloma eta Albertori ere mila esker lurzoru fisikaren ateak zabaltzearren, Hohenheim unibertsitateko biologia saileko guztientzat nire esker ona lurzoru biologiaren mamia erakustearren, eta Macaulay Institute-ko Clare Cameroni eskerrak nire arazo guztiak konpontzeko beti prest egotearren.
Honelako bideetan, egunerokotasuna batzutan gogorra izaten da, eta nire bulegokokideek eta baita laborategikideek ere pisu hau arintasunez eta sarri alaitasunez eramaten lagundu didate. Horregatik, mila esker Oscar, Ainara, Lur, Iker, Goio, Arrate, Aitor, Lidia eta Estiri batik bat, nahiz eta ziur beste hainbeste ahazten ditudan. Baina, Fernandok egunerokotasuna baino beste gauza batzuk ere erraztu izan dizkit eta bere izateko era berezi horrek ikuspuntu ezberdinak ulertzen lagundu izan dit, dena ez da beltza edo zuria, ezta Fernando?
Baina mendiko botek egin dizkidaten babak eta eguzkiak egin dizkidan erredurak osatzen lagundu didatenak etxekoak izan dira. Aita, ama, Aiora eta Axier, eskerrak zauri guzti horiek osatuta dauden gaur, nahiz eta orbain bat baino gehiago utzi duten. Eta noski, Haritz zu niretzat etxekoa zara, baba bat baino gehiago osatu duzulako. Zuek, nik bezain besteko poztasuna sentitzen duzue helmugara iritsi garen egun honetan. Baina hitzez adierazi ezin dudan eskerrona zuretzat da Aitzol, sarri nire pisu osoa eutsi behar izan duzulako eta beti zutitzen lagundu izan nauzulako. Beraz, gaur denontzat da kamiseta, lortu dugu!

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1. Introducción

1.1. Background

La conferencia de las Naciones Unidas sobre Medio Ambiente y Desarrollo Sostenible celebrada en Río de Janeiro en 1992 conocida como *Cumbre de la Tierra* hizo hincapié en que el desarrollo sostenible y la gestión sostenible del medio ambiente marchaban a la par. La Declaración de los principios para la ordenación sostenible de los bosques, sin fuerza jurídica obligatoria, constituyó el primer intento global en el logro de criterios para el consenso sobre el manejo, conservación y desarrollo sostenible de los bosques.

En relación a esta declaración, las naciones se comprometieron a promover la gestión forestal sostenible en sus países. Esto incluye la identificación y la monitorización de funciones ecosistémicas relacionadas con la producción, biodiversidad, capacidad de regeneración, vitalidad y protección de suelo y agua, siendo objetivo de la sostenibilidad el mantenimiento de estas funciones ahora y en el futuro a escala local, nacional y global, sin causar perjuicios a otros ecosistemas (MCPFE, 1993).

Basados en este compromiso, el instituto europeo del bosque cultivado (IEFC), en colaboración con los diferentes gobiernos regionales, centros de desarrollo regional y centros de investigación, inicia un programa de investigación en 2003 titulado Red de zonas piloto para evaluar y mejorar a nivel regional los indicadores de gestión forestal sostenible en los bosques atlánticos del sur de Europa (FORSEE) (*http://www.iefc.net*).

El objetivo era el estudio interdisciplinar de las metodologías y capacidades para la evaluación, seguimiento y la promoción de la gestión sostenible de los bosques del sur de Europa. Uno de los objetivos específicos de esta investigación era la aportación de nuevos conocimientos para la mejora de indicadores de gestión forestal sostenible adaptados a las necesidades de las diferentes regiones.

En el País Vasco, la gestión forestal intensiva de plantaciones de coníferas puede incidir fuertemente en los ecosistemas edáficos, por lo que se planteó un estudio específico sobre la gestión del suelo. El suelo es un recurso no renovable que soporta el desarrollo de la vegetación, provee nutrientes, agua y aire a las raíces de las plantas y determina fuertemente la productividad. La productividad es un componente básico de los ecosistemas, que condiciona su funcionamiento y el conjunto de bienes y servicios que puede proveer. Condiciona así su dinámica natural y su respuesta a los modelos de gestión. Por ello, el uso sostenible del suelo es un elemento central de la gestión forestal sostenible.

La estrategia temática europea para la protección del suelo considera la materia orgánica del suelo como factor clave en el mantenimiento de las funciones del suelo a largo plazo, ya que cumple

funciones esenciales en la estructura y estabilidad del suelo, en su fertilidad y su capacidad de retención de agua, además de aumentar su resistencia a la erosión y de contribuir a evitar la contaminación de los cursos de agua, gracias a su papel de filtro natural (EC, 2006).

El trabajo presentado en esta tesis es parte del estudio específico del FORSEE focalizado en la caracterización de la estructura y dinámica de la materia orgánica en ecosistemas forestales seminaturales y cultivados, la evaluación de los efectos de las prácticas silvícolas intensivas en las diferentes fracciones de la materia orgánica y la exploración de nuevos parámetros como indicadores sensibles de los cambios en el funcionamiento del los suelos. La tesis se titula: "*Estructura y dinámica de la materia orgánica en ecosistemas forestales templados: de lo particular a lo general"*.

1.2. Los bosques de zonas de clima templado del País Vasco

El País Vasco se caracteriza por tener dos regiones biogeográficas; en el norte la eurosiberiana y en el sur la mediterránea. La zona eurosiberiana, que coincide con la vertiente atlántica del país, es un territorio bajo un clima templado húmedo según la clasificación de Koeppen-Geiger-Pohl, situado en el extremo sur del bioma de los bosques templados. Presenta unas condiciones climáticas de extrema atlanticidad con veranos poco calurosos e inviernos poco fríos (la variación anual es de 8 y 13 ºC) y unas altas precipitaciones (900-2000 mm) que se distribuyen por todas las estaciones del año con un mínimo en verano que no llega a causar un déficit severo de agua o sequía. La constante entrada de borrascas atlánticas, con sus frentes asociados, y la proximidad de la corriente del Golfo de Bizkaia que caldea las aguas, determinan las condiciones básicas que gobiernan el clima de la zona templada del País Vasco. Es un clima con poca severidad.

Este clima que permite aproximadamente 6 meses de estación de crecimiento desarrolla una vegetación natural dominada por bosques de robles y hayas con notas puntuales de encinas o marojos (Aseginolaza et al., 1996). Los bosques de roble pedunculado (*Quercus robur* L.) pueden ser acidófilos o éutrofos y se desarrollan en todo el piso colino, hasta 600 m de altitud. Tienen una transpiración elevada por lo que requieren una precipitación superior a 600 mm anuales de los cuales 200 mm han de ser en verano. El robledal acidófilo que ocupa preferentemente suelos de ladera comparte su presencia con el castaño (*Castanea sativa* L.) cediendo el paso a la aliseda (*Alnus glutinosa* L.) en los fondos de valle. El robledal éutrofo, por otro lado, ocupa las vegas de los valles con suelos profundos, ricos en nutrientes y que se encharcan temporalmente. Los robledales comparten el piso colino con los encinares (*Quercus ilex* L.) que siendo bosques típicamente mediterráneos destacan puntualmente en los valles atlánticos. El encinar cantábrico es un relicto del terciario, con climas muy próximos al del litoral mediterráneo actual. Sin embargo, en épocas desfavorables se ha acantonado en los ambientes más próximos a aquellas condiciones y donde no ha encontrado competidores: laderas calizas soleadas con fuertes pendientes y suelos esqueléticos, secos por escorrentía y fácil drenaje. La zona baja del piso montano (600-900 m) por otra parte, es pleno dominio del hayedo (*Fagus sylvatica* L.), acidófilo sobre montañas silíceas y calcícola sobre montes calizos. Las hayas requieren además de una elevada pluviosidad una humedad ambiental también elevada por lo que en sitios menos propicios son sustituidos por el marojo (*Quercus pyrenaica* L.).

Sin embargo, para satisfacer la demanda de las poblaciones, la mayor parte de los países templados incluido el País Vasco han modelado su paisaje convirtiendo sus bosques naturales a usos agrícolas, a plantaciones forestales productivas, a viviendas o a infraestructuras. Actualmente, las plantaciones de coníferas y en particular de pino insigne juegan un papel de

primer orden en la zona de clima templado del País Vasco. Según el Inventario Forestal Nacional del 2005 el pino insigne ocupa alrededor de 140.000 ha en la vertiente atlántica de la Comunidad Autónoma del País Vasco, es decir, el 55 % de la superficie forestal de dicha vertiente. Los bosques de vegetación natural tales como el roble o el haya han sido relegados a enclaves dispersos y ocupan actualmente 20.000 y 15.000 ha respectivamente (IFN, 2005).

1.2.1. Características edáficas de los bosques de zonas de clima templado del País Vasco

El suelo es un medio heterogéneo y dinámico que acoge una gran variedad de nichos ecológicos que difieren en propiedades físicas, químicas y biológicas. Sus características y propiedades dependen de la acción de agentes abióticos y bióticos actuando sobre un material litológico, acondicionado por el relieve y drenaje durante un periodo de tiempo. Los componentes primarios del suelo son (1) compuestos inorgánicos, principalmente aluminosilicatos, oxi-hidróxidos de Fe, y carbonatos, (2) materia orgánica viva y muerta, raíces de las plantas y la biomasa de microorganismos y animales, y residuos orgánicos en diferentes fases de descomposición, (3) disolución del suelo y (4) una fase gaseosa (Killham, 1994). El material litológico puede estar presente debajo del perfil como lecho rocoso (suelo residual) o puede ser originario de otro sitio distinto al lugar donde se ha formado el suelo (suelo alóctono), estos últimos suelen ser muy habituales en zonas montañosas con fuertes pendientes como la zona atlántica del País Vasco.

Los procesos que forman el suelo a partir de este material litológico son (i) meteorización (alteración del material original) y (ii) edafogénesis (reorganización de los constituyentes del suelo), siendo el agua uno de los factores más importantes que incide en dichos procesos formativos (Camps-Arbestain et al., 2008). Dependiendo del comportamiento del agua se diferencian tres tendencias de evolución de suelos: la tendencia ácida en condiciones percolantes, la tendencia alcalina en ambientes con un déficit de agua debido a la elevada evapotranspiración y la tendencia reductora en condiciones anaeróbicas (Chesworth, 2000). Dentro de la tendencia ácida, los procesos de meteorización químicos predominantes en zonas de clima templado húmedo son acidólisis, hidrólisis y descarbonatación (Macías y Chesworth, 1992).

Sin embargo, en este punto, el suelo forestal tiene unas características propias ya que la vegetación arbórea incide en los procesos formativos del suelo mediante la actividad biológica. La actividad radicular de los árboles ejerce una importante acción de meteorización física sobre el material litológico para extraer los nutrientes necesarios para su ciclo vital. Además, en los suelos forestales, la capa de mantillo (Horizonte O) desarrollada gracias a la densa cubierta vegetal y el clima templado y húmedo cobra especial importancia ya que incorpora la mayor parte de los compuestos orgánicos para los procesos edafogénicos del suelo y regula el régimen hídrico y

térmico gracias a su mulch natural. El mantillo desarrollado sobre los suelos forestales de este clima puede clasificarse como mor, moder o mull según el grado de interacción organo-mineral y la diversidad biológica (European Humus Research Group, 2004). En líneas generales, el mantillo Mull (horizontes OL y OF) se caracteriza por tener un pH más alto que moder o mor por lo que favorece un pH superior a 4.2 en el suelo. Estos valores por otra parte, favorecen la presencia de la comunidad de lombrices que tiene una función incorporadora y mezcladora de la materia orgánica y el suelo mineral. Bajo estas condiciones, se forma un horizonte Ah rico en materia orgánica y complejos organo-minerales primarios (arcilla-humus) y secundarios (agregados) y desaparece el horizonte OH del mantillo. La transición entre el horizonte orgánico y el suelo mineral suele ser gradual. Al contrario, el mantillo Mor se caracteriza por su alto contenido de ácidos orgánicos (e.g. ácidos fenólicos) por lo que suele ser habitual encontrar valores de pH inferiores a 4.2 en el suelo y por debajo de estos valores la mayoría de los organismos evitan el contacto con el suelo mineral por sus propiedades tóxicas (Graefe et al., 2002). De esta forma, Mor se caracteriza por desarrollar un horizonte donde se acumulan los residuos vegetales sin descomponer con escasa presencia de animales (horizontes OL y OM) y una transición abrupta entre el horizonte orgánico y el suelo mineral. El mantillo Moder presenta unas características intermedias, presenta una acumulación de la materia orgánica por la menor actividad de la fauna y la biomasa microbiana es predominantemente fúngica por unas condiciones más ácidas que en el mull que genera una biomasa microbiana predominantemente bacteriana (horizontes OL, OF y OH) (Ponge et al., 2003). Comparándolo con mull y moder, la conservación de la materia orgánica en mor está en su máximo y la disponibilidad de nutrientes en su mínimo (Aerts, 1995). Los mantillos mull se encuentran principalmente en pastos y bosques caducifolios con un sotobosque muy diverso, los moder principalmente en bosque caducifolios y coníferas con un sotobosque menos diverso y los mor en prados de montaña con ericáceas. Sin embargo, algunas coníferas pueden desarrollar un mantillo mor principalmente en severas condiciones climáticas (Northup et al., 1998) o cuando han sido introducidos como especies exóticas (Nihlgard, 1971).

Tabla 1.1: Clasificación de mantillo forestal,
donde + significa presencia, - ausencia y ± significa presencia o ausencia.

Mantillo	OH	Ah	arcilla-humus	transición	Fauna
Mull	-	+	+	Gradual	+ (lombrices, otros animales)
Moder	±	+	-	No abrupto	+ (artrópodos)
Mor	±	±	-	Abrupto	-

La amplia combinación de estos factores ambientales determina que los suelos predominantes en la zona forestal atlántica sean suelos ácidos muy jóvenes clasificados como Leptosoles,

Regosoles, Cambisoles y Luvisoles con mantillos mor, moder o mull según la especie forestal y la materia parental (FAO, 1998; Soil Survey Staff, 2006). Los Leptosoles y Regosoles son muy comunes en zonas montañosas donde los procesos erosivos naturales marcan los procesos formativos del suelo, son suelos muy delgados de escasa evolución. Los Leptosoles se desarrollan sobre materiales sílicicos ácidos (Ranker) o carbonatados (Rendzina) coherentes y duros, y presentan habitualmente un perfil tipo AC. Los Regosoles también presentan un escaso desarrollo con un perfil tipo AC con una variación gradual de la materia orgánica en el perfil, aunque no suelen ser tan delgados como los Leptosoles. Al contrario, los Cambisoles y Luvisoles se caracterizan por una mayor evolución y una diferenciación de un horizonte cámbico en Cambisoles (brunificación) y árgico en Luvisoles (eluviación/iluviación de arcillas), por lo que presentan habitualmente un perfil ABC.

1.2.2. Gestión forestal en la zona de clima templado del País Vasco

En la historia forestal del País Vasco se diferencian dos modelos de gestión forestal; la tradicional y la intensiva reflejando dos épocas bien diferenciadas. La **gestión forestal tradicional** practicada desde la Edad Media hasta el siglo XVIII, armonizaba diferentes tipos de gestión para abastecer las diferentes necesidades de la sociedad de la época (Loidi, 2005). El monte bajo de recepe se utilizaba para conseguir piezas pequeñas para leña y carbon, siendo muy habitual el monte bajo de castaño para cestas de pescadores. Sin embargo, la dasotomía de trasmochos, bien de roble o de haya (Figura 1.1), permitía el libre pastoreo del ganado además de satifacer la necesidad de piezas pequeñas para leña y carbón vegetal. En este modelo de explotación forestal los árboles eran descabezados a 3 metros de altura, el espacio de tiempo entre cosechas era de 8-10 años y cada vez se cosechaban la mitad de las ramas (Figura 1.2). El trasmochado permitía a su vez obtener grandes piezas curvadas para la construcción naval cada 40 años en vez de cada 100 años como en los montes altos (Aragón, 2001). Además de la madera, la sociedad de la época extraía brozas como medio indispensable de obtener abono para sus cultivos y material para camas de ganado. Esta práctica generalizada incluía argoma, helecho en calveros y la hojarasca.

Pero en el siglo XIX, los montes públicos sufrieron una importante privatización como consecuencia de la venta de bienes para pago de deudas contraídas por las entidades públicas, principalmente a causa de guerras que afectaron al País Vasco (Ruiz Urrestarazu, 2001). Los nuevos propietarios forestales explotaron sobremanera los recursos forestales para aprovechar la gran demanda de carbón vegetal para la industria del hierro de la época (por ejemplo, para obetener 100 kg de hierro se necesitaban 500 kg de madera de haya, roble o encina), lo que consiguió que en 1872, no existiera en toda Bizkaia ningún monte nacido de semilla y sin las

copas sin desmochar (Michel, 2003). Además, los bosques sufrían una crisis funcional por la morfología de los árboles, tenían una escasa regeneración y estaban sometidos a servidumbres. Estos hechos dieron paso a una alternativa a la actividad forestal tradicional en crisis.

Figura 1.1: Robles trasmochos (izquierda) y hayas trasmochas (derecha).

La **gestión forestal intensiva** se empieza a practicar a partir del siglo XX con el cultivo de especies exóticas con un carácter protector en la lucha contra la erosión de las cuencas más deforestadas. Tras varios ensayos con diferentes especies exóticas se concluye que el pino radiata es una especie muy productiva (9-11m^3 ha^{-1} $año^{-1}$) y rentable (325 pesetas ha^{-1} $año^{-1}$, 12 % de tasa interna de retorno) adecuado para el piso colino de la zona de clima templado del País Vasco (Adan de Yarza, 1916). El forestalismo de inicios de siglo XX era individualizado y con un planteamiento no excluyente, ya que el agricultor integró los pinares en el modelo productivo del caserío y ejerció de agricultor-ganadero-selvicultor.

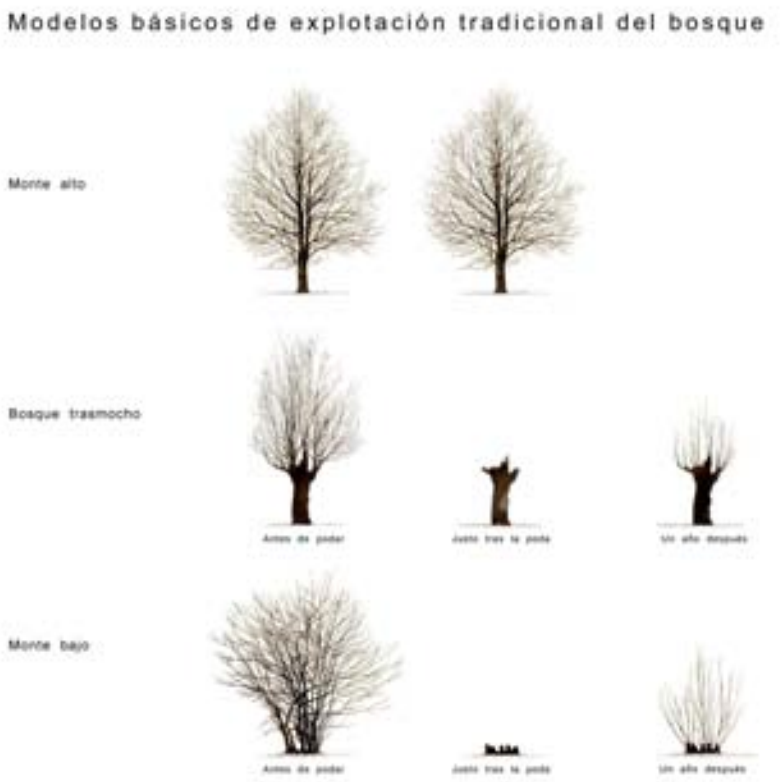

Figura 1.2: Los dos tipos principales de explotación del monte: monte bajo y monte trasmocho: 1 antes de la corta; 2 inmediatamente tras la corta; 3 un año después de la corta. Cedido por Prof. Dr. J. Loidi.

Sin embargo, a medida que abandonaban parte de la actividad agraria debido a la industrilización y el crecimineto demográfico, las praderas y pastizales que se quedaban libres se repoblaban con pino radiata. De esta forma, el paisaje que cumplía funciones productivas y protectoras se transforma en un paisaje monótono de monocultivos de pino radiata con un modelo intensivo y monetarista (Groome, 1987). A partir de los años 80, con intención revalorizar el monte productivo el Gobierno Vasco considera que es necesario mejorar las técnicas de producción forestal (Goicoechea, 1986) incrementando las intervenciones en las masas forestales: plantaciones, limpias, podas, tratamientos, entresacas, cortas y construcción de vías de saca, siendo este modelo de silvicultura el que se sigue practicando actualmente (Figura 1.3).

Figura 1.3: Gestión forestal intensiva en un rodal de pino radiata (Mesa Intersectorial de la Madera, 2002).

1.2.3. Efecto de los distintos tipos de gestión forestal en el sistema edáfico

El suelo es un recurso natural no renovable a escala de vida humana por lo que algunas actuaciones en el sistema pueden tener efectos irreversibles. Respecto a la conservación del suelo la gestión forestal tradicional y la intensiva difieren principalmente en: i) cosecha parcial vs. matarrasa, ii) regeneración natural vs. preparación del terreno y plantación, iii) utilización de fertilizantes y fitosanitarios en la gestión forestal intensiva y iv) bosques de especies mixtas con especies autóctonas vs. monocultivos de especies exóticas o genéticamente modificados.

La gestión forestal tradicional practicada hasta el siglo XVII suponía la tala y entresacado regular de árboles, pero garantizaba también una cobertura permanente del suelo y la continuidad del ecosistema mediante regeneración natural, ya que limitaban su uso a niveles que podían ser compensados por la regeneración. Se podría decir que se hacía un uso sostenible de los recursos forestales. Sin embargo, gracias al incremento de la demanda de carbón vegetal de los siglos XVII-XVIII, los bosque se vieron sobreexplotados, y aunque se establecían plantaciones de robles

y hayas para equilibrar esta explotación, no se consiguió disminuir la erosión por deforestación y ni la exportación de nutrientes mediante cosecha de árboles, hojarasca, helechos o argoma. Además, para la regeneración natural se vió limitada por el sistema de libre pastoreo del ganado que existía en el País Vasco.

Sin embargo, con la revolución industrial que se dió tras la Guerra Civil, el carbón vegetal producido en los montes no era suficiente para abastecer la demanda y la energía utilizada en la siderurgia pasó a ser el carbón mineral. De esta manera, la gestión de los bosques naturales desde aquella época se reduce al libre pastoreo del ganado.

La gestión forestal intensiva que habitualmente se viene practicando en el último cuarto del siglo XX incide más fuertemente en el sistema edáfico ya que las actuaciones en el rodal son menos espaciadas. Los riesgos más reconocidos por la comunidad científica están relacionados con la pérdida de suelo y la depleción de nutrientes (Martínez de Arano et al., 2007a).

La **pérdida de suelo** está ligada principalmente a la corta a hecho o matarrasa, la construcción de pistas y vías forestales, y la preparación mecanizada del terreno. La matarrasa elimina la cubierta vegetal arbórea y daña considerablemente la vegetación de estratos inferiores (Merino et al., 1999). Sin embargo, en regiones de montaña como la zona atlántica del País Vasco, las pistas y vías forestales son una de las principales generadores de sedimentos en la actividad forestal (Megahan, 1980). La cantidad de sedimentos generados por corrimientos de tierras en un evento de tormenta puede variar entre 40 y 8000 t por km de vía (Fransen et al., 2000). La preparación del terreno que tiene como objetivo *limpiar* el terreno para eliminar la competencia de la vegetación espontánea, así como facilitar las labores de plantación, consiste en la eliminación de la vegetación existente, así como en el tratamiento de los desechos tanto de la corta anterior como los generados en la propia limpia. Esta labor puede ser manual (desbroce) o mecanizada (roza al aire con o sin subsolado). Estudios de evaluación directa de la perturbación del suelo han mostrado que las labores mecanizadas producen típicamente alteraciones del suelo en el 80% de la superficie, básicamente por decapado, compactación y desplazamiento de suelo (Martínez de Arano et al., 2007a).

La **depleción de nutrientes** está relacionado principalmente con la pérdida de materia orgánica (MO). La extracción de la biomasa árborea puede disminuir la cantidad de nutrientes disponibles en el suelo (Jhonson y Todd, 1998; Olsson et al., 2000), sobre todo si los restos de corta no se dejan en el monte. Además de la pérdida de nutrientes por biomasa, las actividades de cosecha y preparación del terreno pueden producir una remoción del mantillo en más del 80% de la superficie (Martínez de Arano et al., 2007a) y la lixiviación de nutrientes tras la corta a hecho puede incrementarse, siendo de especial importancia en suelos ácidos (Merino et al., 2004).

Por lo tanto, el efecto de estas actuaciones en el sistema edáfico puede incidir en el buen funcionamineto del mismo, disminuyendo la fertilidad del suelo, incrementando las exportaciones de nutrientes y sedimentos a los cursos de agua y reduciendo el reservorio de C, es decir pueden poner en entredicho la sostenibilidad del suelo (Nambiar, 1996).

1.3. La sostenibilidad de los suelos forestales

Los suelos desarrollados bajo bosques naturales son el patrón de referencia tanto en términos funcionales como en términos de naturalidad e integridad ecológica. Las especies ocupan el lugar y se desarrollan en función de la capacidad del suelo para aportar agua y nutrientes y estos se conservan en un ciclo eficiente suelo-planta intermediado por la materia orgánica. La exportación de sedimentos hacia los cursos de agua es generalmente bajo puesto que los sistemas forestales naturales se caracterizan por presentar una alta cobertura vegetal y una alta capacidad de intercepción de la precipitación, un mulch natural de hojarasca y un buen desarrollo del horizonte superficial del suelo, relativamente rico en materia orgánica y bien estructurado (Pritchett, 1987). Todo ello, favorece la infiltración y reduce tanto la escorrentía superficial, como el impacto directo de las gotas de lluvia sobre el suelo, por lo que se reconoce su papel en la regulación del ciclo hidrológico, así como en la mitigación de la erosión y de la exportación de sedimentos a los cursos de agua (Morin et al., 1981; Ben-Hur et al., 1992; Franzluebbers, 2002; Lado et al., 2004). Los suelos, por otra parte, son el nicho de una substancial, si no de la mayor porción de la biodiversidad de los ecosistemas terrestres (Bauhus et al., 2002).

Por lo tanto, mantener o incrementar la capacidad del suelo para proveer de esas funciones debería ser uno de los objetivos primordiales del uso sostenible del suelo, es decir la mantener o incrementar la **Calidad del Suelo**.

1.3.1. Fertilidad, Calidad y Salud del Suelo

La Calidad del Suelo no es un término nuevo (Warkentin y Fletcher, 1977), aunque tradicionalmente se ha relacionado con el mantenimiento de la productividad a lo largo del tiempo (Carter et al., 1997), por lo que mantener la calidad del suelo era sinónimo de mantener la fertilidad. Sin embargo, el reconocimiento global de la necesidad de entender el suelo de una manera holística, en un marco de calidad medioambiental, ha ampliado la definición del término Calidad del Suelo, que actualmente se entiende esencialmente como la capacidad del suelo para mantener todas sus funciones (Larson y Pierce, 1991; Doran y Parking, 1994; Karlen et al., 1997). Una definición más amplia de la calidad del suelo se refiere a su capacidad para sostener el desarrollo de la vegetación, su habilidad para actuar como filtro y proteger otros compartimentos

del ecosistema, notablemente las aguas continentales y la calidad del aire, para contribuir a regular los ciclos globales de los elementos y para salvaguardar la salud animal y humana (Harris, 1996). Ligado al concepto de Calidad del Suelo aparece habitualmente el concepto de Salud del Suelo que aunque es equivalente al primero, no siempre es considerado como su sinónimo (Doran y Parkin, 1994). Numerosos autores emplean simultaneamente los dos conceptos y hacen referencia a calidad/salud del suelo (e.g., Harris y Bezdicek 1994). Sin embargo, la calidad podría hacer referencia a la capacidad intrínseca que tiene el suelo para desempeñar las funciones anteriormente citadas y que díficilmente se vería afectada por el manejo ya que dependen de los factores formadores del suelo (materia parental, clima, vegetación y relieve) y la salud podría hacer referencia a las propiedades más dinámicas que podrían versse afectadas por el cambio de uso o manejo (Carter, 1997). Generalmente, cuando se hace referencia al estado del suelo o cambios del mismo debido a una actividad se utiliza el término calidad, aunque quizás se trate de la salud del suelo. Hoy por hoy, la comunidad científica mantiene abierto el debate sobre la definición y aplicación del concepto "Calidad de Suelo" frente al de "Salud del Suelo". De cualquier manera, en este trabajo, utilizaremos "Calidad del Suelo" en el marco de la definición de Harris (1996).

En cualquier tipo de sistema edáfico, la "Calidad del Suelo" puede mantenerse, degradarse o mejorar (Karlen et al., 2003) y algunas actividades forestales pueden favorecer su degradación mientras otras pueden contribuir a su mantenimiento o incluso su mejora, por lo que la Sostenibilidad Forestal debe ir ligada al mantenimiento de la Calidad Medioambiental del que es parte intrínseca la Calidad del Suelo (Figura 1.4).

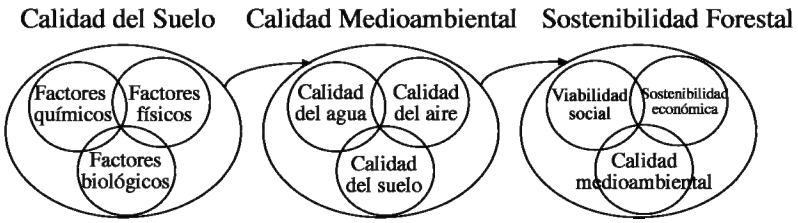

Figura 1.4: Esquema de los diversos factores englobados en la sostenibilidad forestal.

1.3.2. Indicadores de Calidad del Suelo

En el control y seguimiento de la Calidad del Suelo es necesario definir y fijar indicadores y valores de referencia que nos permitan comparar su evolución a lo largo del tiempo así como el impacto que la gestión puede tener sobre ella y su recuperación tras cualquier eventualidad. Los indicadores son propiedades del suelo capaces de caracterizar el sistema adecuadamente y ser suficientemente simples para ser eficientemente y eficazmente monitorizados (Dale et al., 2007).

Los indicadores deben ser precoces en la respuesta en el cambio de la calidad del suelo. Larson y Pierce (1991) desarrollaron el concepto de conjunto mínimo de propiedades del suelo (Minimum Data Set, MDS) para ser utilizados como indicadores de calidad de suelo y poder así monitorizarla. Su MDS constaba de una combinación de propiedades físicas y químicas del suelo (Tabla 1.2), pero Doran y Parking en 1996 incorporaron propiedades biológicas a este MDS. Actualmente, todos los MDS integran aspectos físicos, químicos y biológicos del suelo para la evaluación de la calidad del suelo.

El suelo se caracteriza por la gran variabilidad de tipos y usos, funciones y factores de formación del suelo, por lo que los indicadores adecuados para evaluar la calidad del suelo pueden variar de localidad en localidad (Arshad y Cohen, 1992). Por lo tanto, la identificación efectiva de indicadores apropiados para evaluar la calidad del suelo debe considerar los múltiples componentes de la función del suelo.

Tabla 1.2: Conjunto mínimo de propiedades (MDS) de calidad de suelo propuesto por Larson and Pierce (1991) y la aportación de Doran y Parking (1996) en negrita.

Indicador de Calidad de Suelo
Disponibilidad de nutrientes
Carbono orgánico total
Carbono orgánico lábil
Textura
Agua disponible para planta
Estructura del suelo (Densidad aparente, K_{sat})
Resistencia del suelo (Densidad aparente o resistencia a la penetración)
Profundidad del suelo
pH
Conductividad eléctrica
Biomasa microbiana
Respiración del suelo

1.3.3. La Materia Orgánica del suelo: la esencia de la Calidad del Suelo

La Materia Orgánica (MO) del suelo es un factor clave en el mantenimiento de las funciones del suelo a largo plazo y así lo considera la Estrategia Temática europea para la protección del suelo (EC, 2006), ya que influye de manera importante en las propiedades físicas, químicas y biológicas del suelo. La MO cumple funciones esenciales en la estructura y estabilidad del suelo (Tisdall y Oades, 1982; Six et al., 2002), en su fertilidad nutritiva (Reeves, 1997), en su capacidad de retención de agua (Hudson, 1994) y en su resistencia a la penetración por raíces (Zou, 2000), además aumenta su resistencia a la erosión favoreciendo la infiltración del agua (MacRae y Mehuys, 1985; Boyle et al., 1989; Pikul y Zuzel, 1994) y contribuye a evitar la contaminación de los cursos de agua, gracias a su papel de filtro natural asociado principalmente a sus propiedades adsorbentes (Camps-Arbestain et al., 2004). Por otro lado, la MO influye en la actividad

enzimática del suelo (Dick, 1984; Kandeler, 2005) y en la composición de la comunidad microbiana (Grayston y Presscott, 2005).

Por otra parte, la MO acumulada en los ecosistemas forestales representa una parte importante del reservorio total de carbono orgánico (C). Se estima que la biomasa aérea de los bosques constituye entre el 82-86% de todo el C fijado en la biomasa terrestre (Richter et al., 1999) y que los suelos forestales acumulan entre el 70-73 % de todo el C orgánico fijado en el suelo (Birdsey et al., 1993). El stock actual de C orgánico en la biomasa forestal arbolada de la vertiente atlántica del País Vasco se estima en 13.25 Mt C (raíces incluidas) mientras que el stock actual de C orgánico en los primeros 30 cm del suelo se estima en 29.4 Mt C (Ihobe, 2005). Por lo tanto, los ecositemas forestales y en particular lo suelos forestales juegan un papel importante en el ciclo global del C y el cambio climático (IPCC, 2000).

1.3.4. Cantidad y Calidad de la Materia Orgánica

La materia orgánica del suelo incluye la fracción de residuos orgánicos de plantas, animales o microorganismos en diferentes fases de descomposición (Killham,1994). La cantidad de MO o C orgánico presente en un suelo depende, por un lado, del balance entre las entradas mediante hojarasca (aérea y subterránea) y rizodeposición y, por otro lado, de las salidas que se producen principalmente por la liberación de C durante la descomposición (Jandl et al., 2007), así como por lixiviado y procesos erosivos.

La **cantidad** de hojarasca que se incorpora al suelo en un bosque de clima templado puede variar entre 3500 y 11000 kg ha^{-1} yr^{-1} (Diaz-Maroto y Vila-Lameiro, 2006; Kavvadias et al., 2001; González-Arias, et al., 1998; Barraqueta y Basagoiti., 1988), dependiendo factores como la especie o el tipo de suelo. La MO total incorporada al suelo es una mezcla de compuestos orgánicos, principalmente polisacáridos (almidón, celulosa, hemicelulosa y pectina; 50-60 %) y lignina (15-20 %), pero también suele tener proteínas, polifenoles (e.g. taninos), clorofila, cutina y suberina, lípidos y ceras (10-20 %) (von Lutzow et al., 2006). La abundancia relativa de cada uno estos compuestos y las características moleculares de dichos compuestos varían según la especie; es decir, la estructura química o **calidad** de la MO difiere según la especie. Según el estudio de Lorenz et al. (2004) realizado en el bosque Mannheim-Käfertal de Alemania, la hojarasca bajo pinar se caracteriza por tener taninos condensados y lignina formada por monómeros de guaiacyl propano mientras que la hojarasca bajo roble o haya contiene una lignina en la que dominan los monómeros de siringyl y guaiacyl; por otro lado, los taninos son predominantemente hidrolizables en el roble y condensados en el haya.

La descomposición de la MO está principalmente controlada por la comunidad microbiana dado que menos del 5 % de ésta se oxida por una vía estrictamente química (Lavelle et al., 1993). Los procesos microbianos de descomposición de la MO son controlados por la disponibilidad del sustrato, y por las condiciones ambientales como son la temperatura y la humedad (Prescott, 2002). Bajo condiciones ambientales similares, la descomposición de la MO dependerá de (i) la disponibilidad del sustrato, que depende a su vez de la entrada de hojarasca o cantidad de MO, pero también de la accesibilidad física al mismo, (ii) la estructura química de los compuestos orgánicos o calidad de MO, y (iii) los enlaces entre la MO y el suelo mineral (Jandl et al., 2007). Por lo tanto, la descomposición de la MO dependerá principalmente del grado de estabilización de la MO.

1.3.5. Estabilización de la Materia Orgánica

Conviene distinguir el proceso de estabilización de la MO del proceso de acumulación de la MO. La MO se acumula cuando se producen condiciones que inhiben la descomposición de la misma, tales como (1) un exceso de humedad, (2) temperaturas muy bajas, (3) distrofia, (4) presencia de toxinas, etc. Estas condiciones son típicas en Histosoles (suelos orgánicos), pero también se producen en otro tipo de suelos, como es el caso de determinados horizontes O de suelos muy ácidos (e.g., Podzoles, Umbrisoles). Esta MO suele ser muy poco estable — debido a que apenas ha tenido lugar un proceso de humificación —, y es susceptible de descomponerse si las condiciones que inhiben este proceso se invierten. Por otro lado, el proceso de estabilización de la MO se da (1) por la inherente recalcitrancia de los compuestos orgánicos, (2) por la interacción de la MO con las superficies reactivas de la fracción mineral del suelo (e.g., oxi-hidróxidos de Fe y Al, aluminosilicatos) o (3) simplemente por la inaccesibilidad de la MO para los microorganismos (von Lutzow et al., 2006).

(1) La recalcitrancia de los compuestos orgánicos puede ser primaria o secundaria. La recalcitrancia primaria se relaciona a las características moleculares propias de la hojarasca o rizodepositos de cada especie, mientras que la secundaria es el resultado de diversos procesos metabólicos y degradativos (Sollins et al., 1996). Así, los polímeros que contienen anillos aromáticos como la lignina y las moléculas polimetilénicas como las ceras, cutina y suberina son muy difícilmente degradados por la comunidad microbiana; lo mismo sucede con subproductos generados en el proceso de descomposición como los polímeros húmicos (Lutzow et al., 2006). Esta MO se define como MO estabilizada bioquímicamente.

(2) La adsorción de la MO por las superficies reactivas de los aluminosilicatos y los oxi-hidróxidos de Fe y Al y la complejación de la MO con iones metálicos forma complejos organominerales con una tasa de retorno lenta (Hagedorn et al., 2003). Hay diferencias

considerables entre los distintos tipos de aluminosilicatos: así, la MO enlazada con la esmectita tiene una tasa de retorno de 1100 años mientras que el de la MO asociada a la kaolinita es de 360 años, estando estas diferencias relacionadas con la capacidad de intercambio catiónico efectiva (CICe) y la superficie reactiva de las arcillas (Wattel-Koekkoek et al., 2003). La esmectita es un filosilicato 2:1 con una densidad de carga superficial de 600-800 m^2 g^{-1}, a diferencia de la caolinita que es un filosilicato 1:1, con una densidad de carga superficial mucho menor (10-20 m^2 g^{-1}) (Bohn et al., 1979). La mayor capacidad de estabilización de la MO por parte de la esmectita se debe, no sólo a su mayor carga superficial (Saggar et al., 1996), en relación a la caolinita, sino también al hecho de que es una arcilla expandible y, en el proceso de expansión y posterior contracción puede quedar MO atrapada en su interior.

Como se ha apuntado anteriormente, la adsorción de la MO sobre oxi-hidróxidos de Fe- y Al-, así como la complejación de la MO con iones metálicos forman complejos organominerales con una tasa de retorno lenta. Los oxi-hidróxidos de Fe- y Al- pueden formar ligandos con grupos funcionales de la MO, principalmente con grupos carboxílicos y fenólicos, y la formación de complejos organo-alumínicos tiene lugar principalmente en un rango de pH entre 4.3-4.7 (Gu et al., 1994). Por debajo de ese pH la solubilidad de estos complejos aumenta de forma exponencial, encontrándose predominantemente en disolución (Camps Arbestain et al., 2003). Por lo tanto, este tipo de procesos están limitados a suelos ácidos ricos en minerales con grupos protonados hidroxílicos (Shen, 1999). La MO complejada de esta forma se define como MO químicamente estabilizada. Torn et al. (2002) demostraron que la MO estabilizada de esta forma no varía incluso con marcados cambios de uso tras estudiar series de suelo de 150 años en la estepa de Rusia.

(3) La inaccesibilidad de la MO para los microorganismos y enzimas está principalmente relacionado con la agregación o estructura del suelo. La estructura del suelo se refiere al tamaño, forma y organización de sólidos y huecos, continuidad de poros, la capacidad de retener y trasmitir fluidos y sustancias orgánicas e inorgánicas y finalmente, la habilidad de sostener un buen crecimiento y desarrollo radicular (Lal, 1991); por lo tanto la estructura del suelo es una propiedad dinámica del suelo muy relacionada con la MO y con las características de la misma. Los agregados son partículas secundarias que se forman mediante la combinación de partículas minerales primarias (de tamaño arena, limo y arcilla) con sustancias orgánicas e inorgánicas. Existen varias formas de agregación:

- Teoría jerárquica de agregación (Tisdall y Oades, 1982)

  Este modelo de agregación se propuso para suelos minerales de praderas, pero es uno de los modelos más reseñados en la literatura sobre la estructuración del suelo. La MO se une a los minerales de arcilla mediante cationes polivalentes, conocidos como agentes de unión persistentes,

para formar microagregados (0.5-250 μm) y estos microagregados se van uniendo formando agregados de mayor tamaño a través de agentes de unión temporales como son bacterias, mucílagos y polisacáridos. Estos a su vez, se van uniendo formando macroagregados (> 250 μm) gracias a los agentes de unión transitorios que son principalmente raíces e hifas de hongos.

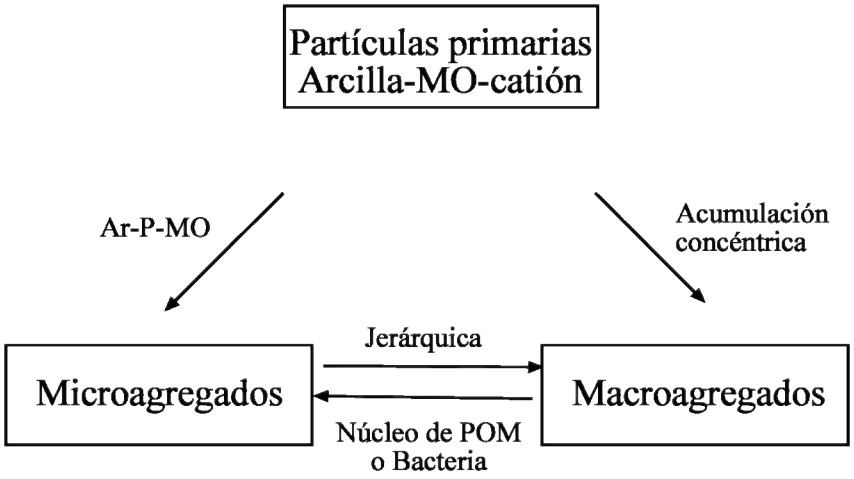

Figura 1.5: Posibles escenarios de agregación del suelo. Materia orgánica (MO), materia orgánica particulada (POM), arcilla (Ar) y particula (P) (Bronick y Lal, 2005).

- Teoría de la acumulación concéntrica (Oades, 1984; Six et al., 2000a; Bronick y Lal, 2005)
  Los macroagregados se forman alrededor de la MO particulada (POM). Cambardella y Elliot (1992) definieron la POM como la fracción entre 2 mm y 53 μm, que consiste principalmente de raíces en diferente estado de descomposición. Los restos vegetales se incorporan al suelo como POM y mientras la POM se descompone y los exudados microbianos son liberados se estabilizan los macroagregados, disminuye la relación C/N y los microagregados se forman en el interior del macroagregado (Fig 1.5).

La MO ocluida en agregados está físicamente protegida contra la descomposición por la reducción de accesibilidad de los microorganismos y sus enzimas, por la reducción de la difusión de los enzimas al espacio intra-agregado y por la reducción de la descomposición aeróbica debido a la disminución de la difusión del oxígeno (von Lutzow et al., 2006).

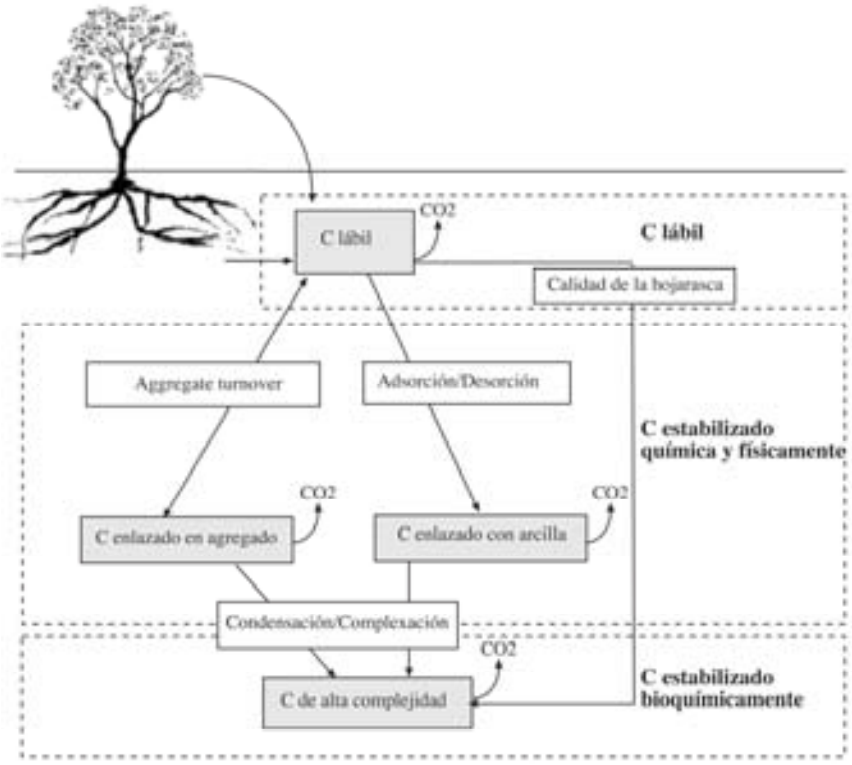

Figura 1.6: Esquema de los procesos de estabilización de la materia orgánica del suelo (Six et al., 2002).

1.3.6. Las fracciones de la materia orgánica como indicadores de sostenibilidad de los suelos forestales.

El contenido total de MO del suelo se puede medir fácilmente por métodos convencionales en diferentes puntos y en tiempos distintos. De hecho, el contenido de MO es un parámetro muy utilizado en muchas redes de monitorización del suelo o inventarios nacionales (e.g., Basonet). Sin embargo, el contenido total de MO del suelo puede responder lentamente a alteraciones medioambientales inducidas por cambios de uso o gestión del suelo, dado que el contenido de fondo de MO es muy grande (Garten y Wullschleger, 1999).

Por lo tanto, la identificación de fracciones de MO más sensibles a la gestión del suelo puede ser útil para dilucidar cambios en la dinámica de la MO del suelo en ecosistemas gestionados intensivamente, ya que nos movemos hacia prácticas más sostenibles. Los modelos que mejor representan el comportamiento de la MO incluyen 2 o 3 reservorios que difieren en su cinética y su tasa de retorno (Parton et al., 1987; Jenkinson et al., 1991). Generalmente, estos reservorios son conceptualizados como uno pequeño con una tasa de retorno rápida y uno o varios reservorios de mayor tamaño y una tasa de retorno mucho más lenta (Cambardella y Elliot, 1992).

Las fracciones de la MO con una tasa de retorno más rápida, de años a décadas, responden habitualmente más sensiblemente a cambios relacionados con la gestión que las fracciones con una tasa de retorno más lentas (Cambardella y Elliot, 1992; Gregorich y Janzem, 1996; Six et al., 1998), por lo que dichas fracciones pueden servir como indicadores o herramientas de verificación de la calidad del suelo y la gestión sostenible, como por ejemplo:

Hojarasca- Los modelos de comportamiento de la MO no consideran la hojarasca del suelo, ya que es sólo característico de suelos forestales. Sin embargo, la hojarasca comprende una de las fracciones más dinámicas de la MO del suelo, por lo que conocer el efecto de las actividades forestales en la dinámica de la MO es crucial para predecir la sostenibilidad de los ecosistemas forestales a escala local y el cambio de C con la atmósfera a escala global (Yanai et al., 2003).

POM- La evidencia de que la materia orgánica tiene un papel importante en la determinación de la agregación está asumida, pero la fracción POM pudiera ser más sensible a cambios en la estructura del suelo por lo que se ha definido como posible indicador de sostenibilidad del suelo (Haynes et al., 1999; Six et al., 2002). El mantenimiento o incremento de la POM como consecuencia de las actividades forestales, podría mejorar la protección física de la MO, la infiltración y reducir la exportación de sedimentos a los cursos de agua.

Comunidad microbiana- La biomasa microbiana representa el 2-3% de la MO del suelo (Kandeler et al., 2005) y se estima que su tasa de retorno es de 0.5-1.5 años (Mc Gill et al., 1986). Sin embargo, esta biomasa es responsable de proveer de nutrientes a las plantas mediante la descomposición de la MO (Bengtson et al., 2007). La descomposición de la MO es un proceso ecológico muy complejo que incluye la interacción de muchos taxones (Sinsabaugh et al., 2002). Hongos y bacterias son los taxones clave en la degradación de la gran cantidad de MO incorporada al suelo todos los años. La composición de la comunidad microbiana depende en gran medida de la cantidad y principalmente de la calidad de la MO incorporada al suelo y la actividad de la temperatura y humedad del suelo (Prescott, 2002).

1.3.7. Perspectiva practica: influencia de las actividades forestales en la MO

Las actividades forestales actuales más importantes en el País Vasco son la utilización de especies exóticas como especies productivas y la cosecha y posterior preparación del terreno mecanizados de dichas especies.

Las características de la cubierta vegetal determinan la cantidad y calidad de hojarasca que influye significativamente en la cantidad de nutrientes a reciclar, en la composición de la comunidad microbiana y por consiguiente en la disponibilidad de nutrientes (Prescott, 2002). Además, las

características de la hojarasca y los subproductos liberados en su descomposición pueden influir en la agregación del suelo (Bronick y Lal, 2005). El diámetro medio de agregado (MWD) y los agregados estables agua (WSA) se correlacionan con la composición bioquímica de los residuos vegetales en cultivos agrícolas. El maíz contiene muchos fenoles en su residuo y una agregación mayor que muchos otros cultivos, al contrario la baja agregación de la soja se relaciona con la baja cantidad de fenoles (Martens, 2000). La actividad radicular también puede influir en la estructura del suelo, ya que las raíces reorganizan las partículas y liberan exudados que producen alteraciones físicas, químicas y biológicas que afectan a la estructura del suelo. La penetración de raíces en el suelo genera canales, especialmente cuando estas mueren y se descomponen. Dichos canales mejoran el movimiento de gas y agua (Moore et al., 1986). Químicamente, las raíces liberan una gran variedad de compuestos con capacidad adherente como por ejemplo los ácidos poligalacturónicos que pueden estabilizar los agregados incrementando la fuerza de los enlaces (Czarnes et al., 2000). Todo ello puede influir en la comunidad microbiana que a su vez genera más compuestos adherentes que mejoran la agregación. La agregación se incrementa generalmente cuando existe una mayor densidad longitudinal de raíces, cuando se producen mayores asociaciones microbianas y cuando se aumenta la cantidad de glomalina en suelo (Rillig et al., 2002). Sin embargo, diferentes sistemas radiculares tienen efectos diferentes en la estructuración del suelo, ya que difieren en las propiedades de las propias raíces, de sus exudados y de sus funciones (Chan y Heenan, 1999b).

En este contexto, el cambio especie podría suponer un cambio importante en la estructura y la dinámica de la materia orgánica y por consiguiente en la Calidad del Suelo.

Por otro lado, las estrategias de cosecha y la intensidad de la preparación del terreno pueden variar según el tipo de ecosistema forestal. En el País Vasco, la corta a hecho mediante motosierra, limpieza de los fustes en el sitio, retirada de los mismos mediante arrastrador y una posterior preparación del terreno utilizando la pala frontal de un buldózer para realizar la limpieza de los retos de corta y la eliminación de la vegetación competidora de la plantación, y que lleva como apero posterior un rejón subsolador que se introduce en el suelo unos 50 cm con la intención de mejorar la estructura física del suelo, son las operaciones selvícolas más habituales durante la cosecha y el establecimiento de la nueva plantación en las repoblaciones de *Pinus radiata* D.Don. Numerosos estudios han demostrado que las cortas a hecho o matarrasa alteran significativamente el mulch natural de hojarasca (e.g., Lavender et al., 1990; Schmidt et al., 1996). Sin embargo, otros estudios han probado que si la cosecha y preparación del terreno se llevan a cabo con sumo cuidado, el mantillo puede incrementar varios años tras la cosecha, dado que la pérdida de

entradas por la biomasa se puede compensar dejando los restos de cosecha en el monte (Post y Kwon, 2000; Yanai et al., 2003) (Figura 1.7).

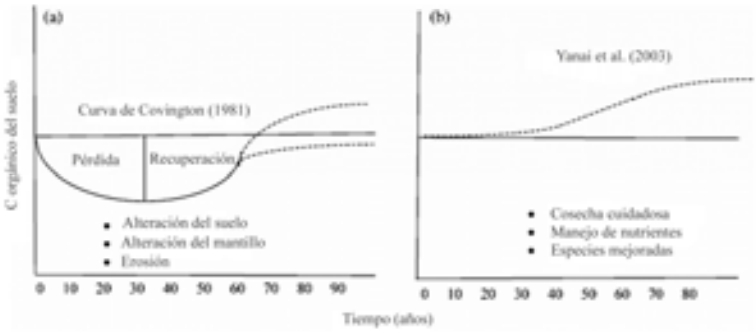

Figura 1.7: Esquema mostrando los efectos de la cosecha y preparación del terreno en el stock de C en suelo (a) con alteración de mantillo y suelo y (b) con alteración mínima del suelo y la incorporación del manejo posible (Lal, 2005).

La preparación mecanizada puede así mismo estimular la decomposición de la MO mediante los cambios producidos en la temperatura y la humedad del suelo (Vitousek y Matson, 1985; Johansson, 1994) culminando en una liberación y pérdida de nutrientes (Merino et al., 2004) y por lo tanto, en una pérdida en la productividad a largo plazo.

Además, la mecanización del terreno influye significativamente a la calidad física del suelo, ya que la compactación del mismo se puede incrementar hasta en un 20 % y la tasa de erosión puede aumentar de 15 kg ha^{-1} $año^{-1}$ a 1.4 Mg ha^{-1} $año^{-1}$ (Martínez de Arano et al., 2007b). La utilización del subsolado que se utiliza en las faenas forestales del País Vasco, podría comparase al arado agrícola aunque sin producirse un volteo de los horizontes del suelo y se ha demostrado que el arado rompe los agregados más grandes del suelo exponiendo a la comunidad microbiana la MO estabilizada en esa fracción, es decir la POM (Elliott, 1986; Cambardella y Elliot, 1992; Six et al., 2000). Los microagregados no se ven afectados por este tipo de gestión ya que se forman por la unión de partículas de suelo a través de agentes de unión orgánicos minerales de tipo persistentes. Sin embargo, los macroagregados pueden ser más sensibles a la gestión y la disrupción de estos agregados puede alterar la dinámica de la MO.

Actualmente, el reto para la comunidad forestal es desarrollar el conocimiento técnico suficiente para implementar modelos de gestión forestal que provean diversas funciones productivas, medioambientales y sociales tanto a la sociedad actual como a la futura y poder validarlos de manrea inequívoca con el conocimiento actual. Los gestores necesitan herramientas de gestión e indicadores sensibles a la gestión propuesta, fáciles de medir y económicamente viables y específicos para cada región, por lo que la investigación básica y comprobación semi-operacional

son necesarios. En este contexto, estudios planteados como "case studies" en ecosistemas forestales representativos pueden ser una forma efectiva de evaluar indicadores potenciales para comprobar la sostenibilidad en la gestión de los suelos forestales y desarrollar propuestas para su aplicación a mayores escalas (Raison y Rab, 2001). Los "case studies" pueden ser particularmente valiosos para definir el nivel de precisión necesario para una correcta representación de las propiedades del suelo para cada tipo de ecosistema forestal. La gran variabilidad natural de estas propiedades puede incrementarse con las perturbaciones derivadas de la gestión, por lo que se necesitarán compensaciones adecuadas entre las medidas detalladas en pocos sitios y las medidas con mayores rangos de incertidumbre para un rango más amplio de sitios.

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2. Objetives

El objetivo general de este trabajo es describir la estructura y dinámica de la materia orgánica (MO) del suelo de ecosistemas forestales presentes en la zona de clima templado del País Vasco, así como evaluar el efecto de la intensificación de la gestión forestal sobre la estructura y dinámica de dicha MO, además de valorar la utilización de diversas fracciones de la MO como indicadores de Calidad del Suelo o de Gestión Forestal Sostenible. Este objetivo general se intenta alcanzar con los siguientes objetivos más específicos:

1. Caracterizar propiedades físicas, químicas y biológicas del mantillo (Horizonte O) en rodales seminaturales y cultivados adyacentes (Capítulo 3).

2. Explorar la relación de diversas fracciones de la MO del horizonte superficial del suelo con diferentes parámetros físicos y químicos en rodales seminaturales y cultivados adyacentes, y estudiar el efecto de la intensificación de la gestión sobre ellos para obtener un conjunto mínimo de parámetros descriptores de los cambios operados en el funcionamiento del suelo (Capítulo 4).

3. Determinar la relación entre cantidad y calidad de la MO y la estructura del suelo de rodales seminaturales y cultivados adyacentes (Capítulo 5).

4. Determinar el efecto de la especie forestal dominante sobre las fracciones más lábiles de la MO en la zona de clima templado del País Vasco a escala de paisaje (Capítulo 6).

The overall objectives of this study were: to describe the structure and dynamics of the soil organic matter (OM) in forest ecosystems in the temperature climate of the Basque Country; to evaluate the effect of the intensification of forest management on the structure and dynamics of the OM, and also to evaluate the use of different fractions of the OM as indicators of soil quality and of sustainable forest management. In order to achieve these overall objectives, the following more specific objectives were considered:

1. Characterization of the physical, chemical and biological properties of the forest floor layer (O Horizon) in adjacent semi-natural and cultivated stands (Chapter 3).

2. Exploration of the relationship between the different fractions of the OM of the surface horizon of the soil and different physical and chemical parameters in adjacent semi-natural and cultivated stands, and of the effect of the intensification of management on these, to obtain a minimum set of parameters to describe the changes in soil functioning (Chapter 4).

3. Determination of the relationship between quantity and quality of the OM and soil structure in adjacent semi-natural and cultivated stands (Chapter 5).

4. Determination of the effect of the dominant forest species on the most labile fractions of the OM in the temperate climate of the Basque Country, at the landscape level (Chapter 6).

3. Characterization of forest floor in adjacent temperate forests: effects of forest activities

The aim of this study was to characterize organic matter in litter (L) and fermented-humified (FH) layers in forest floor under mature beech (Fagus sylvatica L.), pedunculate oak (Quercus robur L.) and radiata pine (Pinus radiata D. Don), which are common in the Atlantic temperate region of the Basque Country. In addition, a chronosequence from two adjacent clear-cut intensively managed pine plantations (3 and 16 years old) was sampled to determine the recovery from the effect of logging operations.

The interspecific differences in forest floor mass and mass distribution were linked to differences in litter quality and decomposition rates, which were in turn related to microbial community. Forest floors under deciduous trees had lower lignin/N, C/N and C/P ratios and higher pH, Ca and Mg contents than those under pine. Microbial respiration was also higher in pine forest, but no differences in microbial biomass and functional diversity in community level physiological profiles (CLPP) were observed. CPMAS ^{13}C NMR and proxymate analysis showed similarities in C functional groups between beech and pine stands.

Forest management induced changes in forest floor mass and decomposition rates. Three years after clear-cutting and following site preparation, forest floor was still absent and 16 years later it was 50% less than in mature pine forest. Microbial respiration rate was higher and microbial biomass and diversity were lower after 16 years of clear-cutting.

The results underline the importance of the type of forest in C transformations and the sustained effects of clear-cutting and site preparation, apparent even 16 years after disturbance.

3.1. Introduction

During the XX century, large-scale plantations of the exotic *Pinus radiata* species were established on abandoned farmland and mountainous land in the temperate Basque Country (northern Spain) (Michel, 2003). This tree species now dominates forest plantations in the region and forests are currently being managed in a clear-cut regime with rotation lengths of between 30 and 40 years, harvesting with chainsaw and skidding, and mechanical site preparation prior to planting by processes such as blading and down-slope ripping. These human-induced changes to the landscape are likely to cause changes in the forest composition, net primary production and consequently, regional patterns of carbon cycling (Pastor and Post, 1988; Finzi et al., 1998; Yanai et al., 2000). Litter decomposition and the subsequent release of nutrients in plant-available forms is an essential process in the functioning of forest ecosystems. The rate of litter decomposition is determined by the prevailing environmental conditions for a given location (Prescott, 1995), by the intrinsic chemical properties of plant litter or litter quality (Almendros et al., 2000), and by the diversity and activity of the microbial community (Leckie et al., 2004). Under similar environmental conditions, the substrate quality and decomposer community, are directly related to biological diversity (Hättenschwiler et al., 2005).

To our knowledge, there are few studies of the effects of forest activities on substrate quality, microbial community diversity and decomposition rates in forest floors in temperate forests. Forest activities in the study region include; (i) introduction of exotic tree species, and (ii) intensive management, with use of heavy machinery for harvesting and site preparation.

Different tree species show different patterns of forest floor accumulation owing to differences in SOM chemistry (Raulund-Rasmussen and Vejre, 1995; Hannam et al., 2004). Quideau et al. (2005) studied Mediterranean evergreen shrubs and found that under scrub oak, leaf litter was dominated by carbonyl C, whereas under manzanita (*Arctostaphylos manzanita Parry.*) litter was dominated by O-alkyl C and under coniferous vegetation, by alkyl C. To our knowledge differences between temperate forests in terms of the chemical nature and distribution of litter organic constituents such as carbohydrates, phenols and carboxylic acids, have not been investigated. These constituents affect the chemical stability of accumulated organic matter and exert a strong influence on soil microbial activity, by functioning as sources of energy for the growth and activity of decomposing organisms (Goh and Heng, 1987).

Despite the differences in accumulation patterns under different tree species, the forest floor of mature forests remains constant because inputs are balanced by outputs. However, disturbances such as clear-cut forest harvesting and subsequent soil preparation, cause drastic alteration of this

balance. Forest harvesting and site preparation change the quantity and biochemical composition of plant litter, which serves as the primary substrate for microbial activity. Abundance of dead organic matter on the soil surface after harvesting and site preparation depend on the forest management practices selected. Stem-only harvesting may leave an abundance of dead organic matter on the soil surface, although inputs of detritus are diminished following this initial contribution. However in forest practices such as blading and subsequent down-slope ripping, all aboveground organic residues are pushed out of the stand with the front blade of a bulldozer (Olarieta et al., 1997; Merino et al., 2004). Furthermore, post-harvest residual organic matter represents a biochemically different substrate from the fine roots, leaves, and dead plant tissues that comprise litter in mature forests (Covington, 1981). Such a change in substrate input has the potential to alter the composition of soil microbial communities because bacteria, actinobacteria and fungi differ in their physiological abilities to metabolize organic substrates contained in plant litter (Paul and Clark, 1996). Borman and Likens (1979) suggested that the rate of nutrient mobilization from the forest floor may regulate recovery after devegetation by insuring the availability of essential elements during recovery. Forest floor plays an important role in recovery after disturbance (Covington, 1981) and the effect of forest harvest on soil organic matter is important not only in terms of local successional processes but also global carbon storage (Yanai et al., 2000).

In this context, the present study was designed to provide basic information on forest floor in temperate semi-natural and cultivated forests. Our lack of knowledge about the chemistry of forest floor organic matter and dynamics means that preliminary studies were required to generate the necessary basic information. The main objectives were: i) to compare the biotic and abiotic characteristics of the forest floors in three stands dominated by different tree species (European beech, pedunculate oak and radiata pine) developed under similar soil parent material, geomorphology and climate, ii) to show the effects on the forest floors of a 3 year old radiata pine stand and of a 16 year old stand, after mechanical logging and site preparation operations, and iii) to explore the relationships among measured characteristics of these forest floors to gain insights into the effect of species change and intensive mechanization on C dynamics.

3.2. Materials and methods

3.2.1. Forest floor sampling

The study site (30T 534075 4783284) was selected as an example of the temperate forest landscape in the Basque Country; the stands in the site had similar soil types (Typic Udorthent, Soil Survey Staff, 2006) and climatic conditions, mean annual temperature and precipitation of

14.1º C and 1200 mm, respectively, and were in close proximity to each other. The strategy of the study was to evaluate three representative stands of two mature semi-natural forests (*Quercus robur* L. and *Fagus sylvatica* L., hereafter referred to as oak and beech and a nearby first-rotation and non mechanized cultivated *Pinus radiata* D. Don plantation (hereafter referred to as pine 40ys) to evaluate the effect of change in tree species on forest floor in these stands. In addition, a chronosequence was sampled from two adjacent clear-cut sites (3- and 16 years old) (hereafter referred to as pine 3ys and pine 16ys) to evaluate the effect of mechanized forest operations 3- and 16-years after disturbance. The logging operations of the two disturbed pine plantations included forest harvesting with skidders and site preparation by blading and down-slope ripping. In blading, the soil surface should be scraped, although sometimes the first centimetres of soil are also excavated and slash and surface organic material are displaced to small piles down-slope (Macdonald et al., 1998); down-slope ripping consists of deep ploughing (≈ 50 cm) following the maximum slope of the stand.

In each stand (areas between 0.8-1.5 ha), three trees were selected at random and forest floor samples (10 samples, 15 x 15 cm template) were systematically collected within a 2.5 m radius from the base of each of these trees on 13th of April 2005. All samples were collected at the same time and were divided into L and FH layers and combined to give one composite sample per tree and layer. The L layer consisted of slightly decomposed litter of identifiable origin, and the FH layer, which we were not able to separate into F and H layers, consisted of partly decomposed and very decomposed organic material. Samples for chemical analysis were oven dried to constant weight, weighed and ground (< 0.5 mm) in a laboratory mill, and samples for microbiological analysis were stored at 4 ºC for no more than 7 days. L layer samples were cut into smaller pieces (≈ 1 cm^2) and FH layers were passed through a 5 mm sieve.

3.2.2. Forest floor chemical, biochemical and moisture analysis

Organic C and total N were analysed in a LECO CNS 2000 (LECO Corporation, MI, USA). Total concentrations of P, Ca, Mg, K and Na were determined in aqueous extracts after wet digestion with nitric-perchloric acid by Inductively Coupled Plasma Emission Spectroscopy (Varian Iberica S.L., Barcelona, Spain). Forest floor pH was determined in 1:10 FF (forest floor):water solution (Blakemore et al., 1977). The water-soluble carbohydrates (WSC) were determined colorimetrically by the anthrone method, in water extracts, according to Deriaz (1961). Acid-detergent fibre (ADF) and acid-detergent lignin (ADL) were determined with an ANKOM^{220} fiber analyzer (ANKOM Technology, NY, USA) (van Soest et al., 1991). The cellulose fraction was estimated as CEL=ADF−ADL and the ADL fraction was considered to be lignin, although it

includes some recalcitrant by-products of decomposition. Ash (ASH) was determined after 4 h at 550 °C. Moisture content of each sample was determined until constant weight at 105 ºC.

3.2.3. Solid-state ^{13}CP MASS NMR spectroscopy

The solid-state ^{13}C NMR spectra of L and FH layers of oak, beech and pine 40 ys were obtained on a Bruker DSX 300 (Bruker Instruments, Karlsruhe, Germany) operating at a ^{13}C resonance frequency of 75.49 MHz. All ^{13}C NMR spectra were acquired by cross polarization (CP). Dry, powdered samples were spun at 4.7 KHz in a 7-mm OD rotor. The ^{1}H 90º pulse length was 4.5 µs, the recycling time was 2 s, and the contact time was 1 ms. Number of scans were around 30000 and the results were processed by 30 Hz line-broadening and baseline correction. Chemical shifts are reported relative to tetramethylsilane (TMS) at 0 ppm, as shown in Table 3.1, which were integrated to determine the percentage of total area (relative intensity).

Table 3.1: Chemical shift assignment of shift ranges for solid-state 13C NMR spectra to C functional groups based on Lorenz et al. (2000).

Shift range (ppm)	Functional group	Abbreviation
0-46	Alkyl C	ALK
45-93	Methoxyl- and O-alkyl carbons	O-ALK
93-112	Di-O-Alkyl C	DI-O-ALKYL
112-140	Aromatic and unsaturated C	AROM
140-162	Phenolic C	PHE
162-184	Carbonyl C-1 (carboxylic acid, ester and amide)	CARB

3.2.4. Forest floor microbial community activity and quantity

Microbial decomposition rates of forest floors were studied in laboratory consisting of airtight glass jars (0.5 L). Three replicates of each sample were weighed (5 g) fresh, and incubated at 28ºC for 28 days. The CO_2 evolved was trapped in 10 ml 1 N NaOH containers and determined on days 1, 2, 3, 8, 14, 22 and 28, by titration with HCl to the phenolphthalein endpoint, after precipitation of carbonates with $BaCl_2$. Carbon mineralization rate or basal respiration (BR) was expressed as microbial respiration at the end of incubation period (µg C-CO_2 g forest floor (FF)$^{-1}$ h^{-1}) and mineralizable C as cumulative microbial respiration during 28 days (µg C-CO_2 g forest floor (FF)$^{-1}$). Microbial biomass carbon was determined by the fumigation-extraction method, which was essentially that of Vance et al. (1987). Briefly, 2 g of fresh forest floor material was fumigated for 24 h at 28ºC with ethanol-free chloroform vapour. Fumigated and unfumigated samples were

extracted with 0.5 M K_2SO_4 (30 min, 200 rpm). The extracts were filtered through Whatman nº 42 filters and total organic C was determined by digestion in chromic acid.

3.2.5. Forest floor microbial community diversity

The functional diversity of microbial communities was characterized by community level physiological profiles (CLPP) with a Biolog Ecoplate™ microplating system (Biolog, Hayward, CA, USA) based on the methods of Degens et al. (2001) and Grayston and Prescott (2005). Ecoplates™ use 31 carbon substrates that are more ecologically relevant compounds and are likely to provide a useful test for microbial analysis and to pick up those microorganisms usually missed through being swamped by faster growing r-strategists species on GN plates (Campbell et al., 1997). Briefly, moist forest floor samples (5 g) were suspended in 50 ml of one-quarter strength Ringer´s solution with 30 glass beads of 3 mm diameter for 15 min in a reciprocating shaker at high speed (250 rpm). Ten fold dilutions were made in de-ionized water, until 10^{-3}, and this was centrifuged at 750 g for 10 min. Aliquots (125 µl) of the resulting suspension were inoculated in Ecoplates™, incubated in dark at 28 ºC and colour formation read as light absorbance at 595 nm after 0, 16, 20, 24, 40, 44, 48, 64, 68, 72, 88, 92, 96 and 160 h in a multifunctional Zenyth 3100 microplate reader (Anthos Labtec Instruments, Salzburg, Austria). The absorbance of the control value was subtracted from the absorbance value of wells containing C substrates for each time. The average number of substrates used (NUS) was calculated on the basis of majority-rules decision established by Goodfriend (1998) whereby substrate use was confirmed if dye reaction was positive (>0.25 absorbance units); the Shannon diversity index was used as practical indicator of overall measure of microbial functional diversity (Degens et al., 2001). Average well colour development (AWCD) of each sample was also calculated and used to transform individual carbon source values to eliminate variation in AWCD caused by different cell densities (Garland and Mills, 1991).

3.2.6. Statistical analysis

One-way analysis of variance (ANOVA) was applied to the forest floor chemical properties to detect differences in the stands dominated by different tree species and differences in the mechanized stands and the non mechanized adult pine stand. Variables were tested for normality and Levene´s test for homogeneity of variances was used prior to conducting one-way analysis of variance, and a protected least-significant difference (PLSD) test was used to determine the significance of main effects revealed by ANOVA. In a similar way, the Student t-test was used to show differences between forest floor layers. These statistical analyses were performed to gain a deeper insight into the data structure. Nevertheless, because of the inherent large variability

usually found in the parameters that characterize forest ecosystems and also the lack of stand replicates, statistical analysis were used to show trends in change among the studied stands, rather than to make specific conclusions about the effects of species change or mechanical operations on forest floor. Differences were therefore also considered significant at $P < 0.10$ (Six et al., 2002).

The CLPP data was firstly analysed by Principal Component Analysis (PCA) to reduce the dimensionality in data. The first 10 components of the PCA were used in a Canonical Variate Analysis (Grayston and Prescott, 2005) to determine whether microorganisms present in the studied forest floor samples differed in the overall patterns of carbon utilisation. The ordination coordinate loadings were also used to identify the carbon sources responsible for the discrimination and component scores were compared by mean separation tests to detect significant differences between tree species (PLSD) and forest floor layer (t-test).

Another Principal Component Analysis (PCA) was also performed, considering the whole standardized data set as an explanatory test of multivariate significance of component factors between groups (Stevenson et al., 2004). Factors in PCA analysis were retained to explain at least 75 % of the variance of the original data and composite variables of the factors were selected on the basis of the variable loadings being greater or equal to 0.6 (Lee, 1993).

3.3. Results

3.3.1. Forest floor mass, chemical and biochemical properties

Total forest floor mass varied with tree species (F= 18.98, $P < 0.01$) and time since disturbance (F= 36.07, $P < 0.001$). Beech stand showed significantly higher forest floor mass (25.2 ± 2.1 Mg ha^{-1}) than pine 40ys (16.35 ± 1.9 Mg ha^{-1}) and oak stands (7.55 ± 0.2 Mg ha^{-1}).

Pine 3ys stand showed a complete absence of the forest floor mainly due to the mechanical site preparation (blading and down-slope ripping) applied on the stand. Pine 16ys stand forest floor (8.13 ± 0.6 Mg ha^{-1}) was higher than in pine 3ys stand but still significantly lower than in mature pine.

One hundred percent (100%) of the forest floor mass under oak was found in the L layer, whereas each forest floor layer accounted for 50 % of the forest floor mass beneath beech. In the two coniferous stands with forest floor (pine 40ys and pine 16ys), the FH layer accounted for 70 % of the forest floor mass in pine 40ys and 30 % in pine 16ys.

In L layers, forest floor pH, and concentrations of Ca, Mg and K were significantly higher in oak and beech stands than in the pine stand (Table 3.2). Total concentration of C was lower in the oak stand than in beech and pine 40ys, however concentrations of N and P were similar in oak and pine

40ys stands and significantly higher in the beech stand. As regards litter quality variables, oak and beech forests showed similar C/N and C/P ratios whereas the ratios in mature pine forest were significantly higher. Pine 16ys and pine 40ys showed similar concentrations of nutrients and C/N ratio, but pine 16ys showed significantly lower C/P ratio in L layer. In FH layers, the only significant differences found between beech and mature pine were the higher pH value and lower C/N ratios in beech forest. Mature pine and pine 16ys stands showed similar chemical properties in FH layers. Forest floor layers differed significantly in concentration of nutrients and quality of organic matter. A significant increase in concentration of potassium, magnesium, sodium and phosphorous and a significant reduction in C/N and C/P ratios was observed with depth (Table 3.2).

The biochemical properties of slightly decomposed litter material (L layer) were similar in beech and pine stands but significantly different in the oak stand. Oak showed significantly lower ADF, CEL and lignin than beech and pine 40ys (Table 3.3). However, lignin/N ratio, another litter quality variable, differed significantly among oak and beech stands and pine 40ys stand, showing highest values in the pine forest. Both pine stands (pine 40ys and pine 16ys) showed very similar biochemical properties in L layer. In FH layers, beech and pine 40ys had similar properties but pine 16ys had significantly higher WSC than pine 40ys. In all stands, ADF, CEL and lignin were significantly lower in FH layer than L layer. WSC also decreased in all forest floors with depth but in pine 16ys (Table 3.3).

3.3.2. Solid-state ^{13}CPMASS NMR spectroscopy

The *^{13}CP NMR* spectra of L and FH layers of mature oak, beech and radiata pine forests are shown in Fig. 1. In forest floor L layers, the ALK region of the spectra showed one mean peak at 29-30 ppm, corresponding to polymethylene type carbons, but oak and beech samples showed an additional shoulder at 19-20 ppm, corresponding to acetate groups. In all tree species, major signals in the -O-ALK region of carbons was found around 73 ppm, which is characteristic of carbons of cellulose and hemicellulose. The shoulder at 63 ppm was assigned to the C-6 carbon of carbohydrates and was noteworthy in beech and oak forest floor L layers. An additional peak at 56-57 ppm was a carbon signal for lignins (Fig 3.1).

In the DI-O-ALK region of the spectra, the C-2 and C-6 carbons of syringyl lignin units probably contributed to the peak centred around 105 ppm (Preston et al., 2000), present in all samples. However, the peak at 117 ppm that may correspond to C-substituted aromatic carbons (Quideau et al., 2005), was less ambiguous in beech forest floor, while phenolic carbons between 140-164 ppm appear more intensely in oak forest floor. Finally, the peak at 174 ppm, which was indicative of carbonyl carbons, was also more noteworthy in oak forest floor.

In the spectra of FH layers, beech and mature pine forests differed mainly in the absence of the peak between 116-118 ppm in the mature pine forest floor, which was indicative of aromatic C carbons. In contrast, the peak of phenolic carbons, between 140-164 ppm, was more intense in pine than in beech. The major differences between forest floor layers were found in mature pine forest with increasing contents of PHE (66% of them in L) and CARB (60% of them in L) in the FH layer. No differences were found between layers in beech stand as differences in these parameters were always less than 80% (Table 3.4).

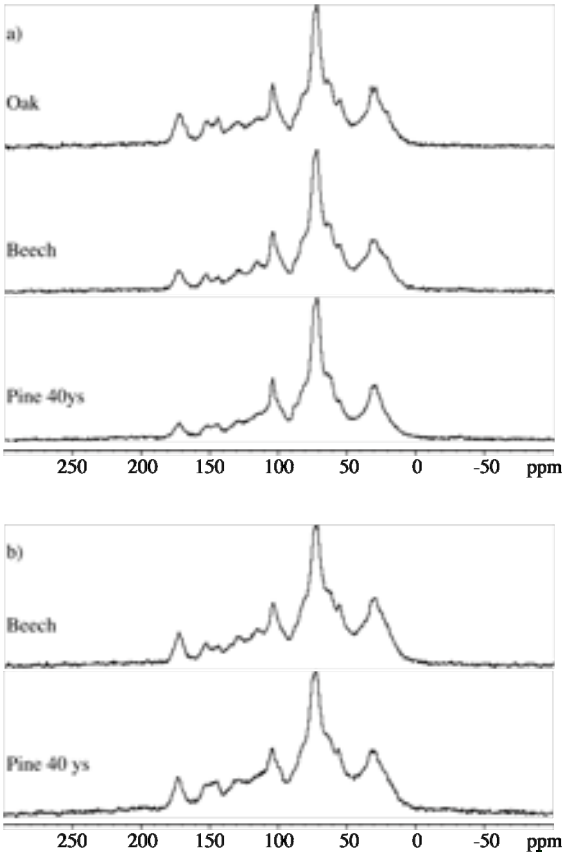

Figure 3.1: CPMASS 13C NMR spectra of a) L and b) FH layer of forest floors from three mature temperate tree species (Oak, Beech and Radiata pine).

Table 3.2: Analysis of variance results for the effects of species, management and depth on chemical properties and moisture content in the stands under study. In the Species section, ANOVA results are presented for stands dominated by different species (Oak, Beech and Pine40ys). In the Management section, ANOVA results are presented for the studied chronosequence (Pine16ys, Pine40ys). In the Depth section, t-Student test results are presented for the forest floor layers (L and FH). Values in the following section are means and standard deviations in parenthesis of these properties. Chemical properties are expressed in mg/g dry-weight basis and moisture content in %.

	moisture	pH	C	N	P	S	Ca	Mg	K	Na	C/N	C/P
Species												
F-value												
L (Df =8)	13.75**	17.34**	6.66*	26.36***	17.58**	21.29**	8.87*	8.52*	10.37*	6.54*	13.54**	5.26*
FH (Df=4)	19.00*	12.43*	ns	ns	7.19*	ns	ns	ns	ns	ns	19.17*	ns
Management												
F-value												
L (Df=4)	ns	ns	ns	ns	41.31**	ns	ns	ns	ns	72.00**	ns	13.23*
FH (Df=4)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Depth												
t-value (Df=8)	-2.04* L<FH	ns	8.84*** L>FH	ns	-3.18** L<FH	ns	5.32*** L>FH	-4.44*** L<FH	-6.54*** L<FH	-5.67*** L<FH	8.20*** L>FH	12.12*** L>FH
Oak												
L	49.72 (0.01)[a]	4.67 (0.10)[a]	349 (33.95)[a]	11.83 (0.86)[a]	0.38 (0.00)[a]	2.22 (0.18)[a]	12.59 (1.74)[a]	1.87 (0.24)[a]	3.75 (0.67)[a]	0.32 (0.03)[a]	29.32 (0.76)[a]	914.82 (77.86)[a]
FH												
Beech												
L	60.06 (0.01)[b]	5.03 (0.05)[a]	483 (2.87)[b]	18.43 (0.29)[b]	0.52 (0.02)[b]	3.47 (0.04)[b]	9.01 (0.28)[a]	1.17 (0.03)[ab]	1.29 (0.01)[b]	0.21 (0.01)[b]	26.23 (0.54)[a]	941.29 (47.25)[a]
FH	65.16 (0.00)[a]	4.67 (0.12)[a]	339 (32.06)	17.10 (1.73)	0.66 (0.06)[a]	3.60 (0.48)	4.68 (0.58)	1.54 (0.11)	3.87 (0.47)	0.34 (0.01)	19.88 (0.40)[a]	516.62 (13.51)
Pine (40ys)												
L	56.26 (0.01)[b]	4.15 (0.10)[b]	472 (22.60)[b]	12.20 (0.47)[a]	0.38 (0.01)[a]	2.93 (0.06)[c]	4.93 (0.46)[b]	0.88 (0.03)[b]	0.94 (0.06)[b]	0.24 (0.00)[b]	38.85 (2.35)[b]	1257.10 (74.06)[b]
FH	58.59 (0.01)[b]	4.04 (0.08)[b]	327 (29.98)	12.17 (0.61)	0.45 (0.02)[b]	3.07 (0.20)	2.84 (0.14)	1.86 (0.27)	5.08 (0.85)	0.42 (0.05)	26.71 (1.14)[b]	730.37 (77.01)
Pine (16ys)												
L	57.42 (0.01)	4.06 (0.04)	493 (2.62)	12.23 (0.54)	0.5 (0.02)	2.95 (0.09)	4.22 (0.60)	0.89 (0.03)	1.42 (0.13)	0.20 (0.00)	40.53 (1.77)	894.45 (33.82)
FH	57.32 (0.02)	4.33 (0.06)	317 (26.54)	11.47 (0.83)	0.59 (0.06)	2.28 (0.00)	2.41 (0.24)	1.49 (0.10)	3.88 (0.35)	0.30 (0.02)	27.59 (0.33)	551.60 (50.26)

Table 3.3: Analysis of variance results for the effects of species, management and depth on biochemical properties in the stands under study. In the Species section, ANOVA results are presented for stands dominated by different species (Oak, Beech and Pine40ys). In the Management section, ANOVA results are presented for the studied chronosequence (Pine16ys, Pine40ys). In the Depth section, t-Student test results are presented for the forest floor layers (L and FH). Values are means and standard deviations in parenthesis and are expressed in % on ash-free dry-weight basis for ASH, ADF, CEL and lignin and in mg/g on dry-weight basis for WSC.

	ASH	ADF	CEL	Lignin	WSC	Lignin/CEL	Lignin/N
Species							
F-value							
LF (Df =8)	ns	5.83**	4.16**	6.04**	ns	ns	6.74**
H (Df=4)	ns	ns	ns	ns	ns	ns	ns
Management							
F-value							
LF (Df=4)	ns	ns	ns	ns	ns	ns	ns
H (Df=4)	ns	ns	ns	ns	209.60***	ns	ns
Depth							
t-value (Df=8)	-8.31*** L<FH	12.06*** L>FH	14.50*** L>FH	10.12*** L>FH	3.02** L>FH	ns	11.04*** L>FH
Oak							
L	10.83 (1.64)	44.02 (4.22)[a]	15.99 (1.25)[a]	28.03 (2.99)[a]	3.12 (0.29)	1.74 (0.1)	23.53 (0.80)[a]
FH							
Beech							
L	5.68 (0.42)	62.17 (0.74)[b]	21.51 (0.68)[b]	40.66 (0.91)[b]	3.55 (0.29)	1.90 (0.09)	22.06 (0.29)[a]
FH	21.58 (2.53)	34.24 (3.67)	10.75 (0.92)	23.66 (3.11)	2.84 (0.11)	2.21 (0.23)	13.80 (0.90)
Pine 40ys							
L	5.91 (1.29)	57.25 (3.47)[b]	20.17 (1.39)[b]	37.23 (2.09)[b]	5.78 (1.39)	1.85 (0.03)	30.70 (2.37)[b]
FH	16.46 (0.84)	34.24 (5.08)	11.66 (1.90)	22.58 (3.19)	3.06 (0.05)	1.95 (0.04)	18.31 (1.64)
Pine 16ys							
L	5.01 (0.34)	57.25 (0.80)	20.80 (0.59)	36.45 (0.22)	5.71 (0.24)	1.76 (0.04)	29.99 (1.44)
FH	23.63 (2.91)	31.74 (2.05)	11.25 (0.94)	20.49 (1.13)	6.65 (0.18)	1.83 (0.05)	17.96 (0.63)

* P < 0.1, ** P < 0.05, *** P < 0.01. Values followed by different letters differ significantly according to the Post Hoc test (Protected Least Significant Difference) for the studied parameter

Table 3.4: Integration of 13CP MASS NMR spectra of forest floor samples for L and FH layers of different tree species.

	ALK	O-ALK	DI-O-ALK	AROM	PHE	CARB
Oak						
L	25.3	48.6	9.8	9.1	3.3	3.9
FH						
Beech						
L	20.5	50.7	11.6	10.0	3.8	3.4
FH	21.9	47.5	11.3	10.8	4.4	4.1
Pine 40ys						
L	20.0	51.4	12.7	9.0	4.1	2.8
FH	20.1	46.6	11.3	11.2	6.2	4.7

3.3.3. Forest floor microbial biomass and C mineralization

In L layers, forest floor microbial biomass carbon did not differ between tree species, but differed significantly in FH layers (Fig 2). Microbial biomass in FH layer was as follows: beech > pine 40ys > pine 16ys. Microbial biomass in the L layer was two times higher than in the FH layer in the beech stand, 3 times higher in pine 40ys and 6 times higher in pine 16ys.

In contrast to microbial biomass, microbial respiration rates of forest floor as indicative of microbial activity differed substantially between tree species in L layers. The release of CO_2 was higher in the pine 40ys stand than in beech and oak stands. However, no differences were detected between older and younger pine stands. Oak and beech stands had 60.2 ± 2.3 and 71.5 ± 10.6 μg C-CO_2 g^{-1} FF h^{-1} respectively, while pine 40ys and pine 16ys stands had 98.8 ± 2.5 and 96.9 ± 3.9 μg C-CO_2 g^{-1} FF h^{-1}. No significant differences were found in microbial respiration rates of FH layers (Fig 3.2). In all stands, microbial respiration rates were significantly lower in the FH layer than in the L layer (t= 6.00, $P < 0.0001$).

The metabolic quotient (qCO_2 is the ratio C-CO_2 respiration to microbial biomass C) did not differ with tree species nor between older and younger pine forests in the L layer forest floor. However, the stand that was clear-cut 16 years prior to the study, showed significantly higher qCO_2 than mature pine stand in FH forest floor layer. The two mature beech and pine forests showed similar metabolic quotients in both forest floor layers (Fig 3.2).

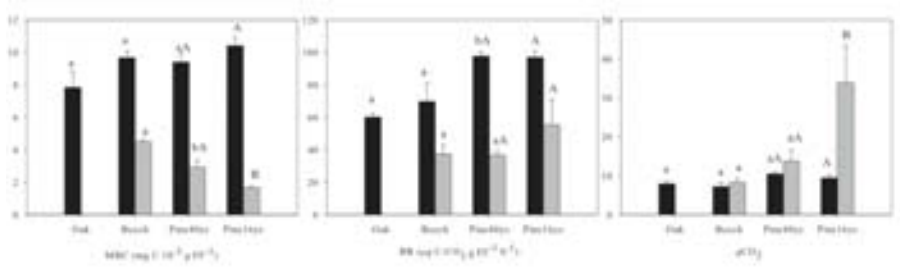

Figure 3.2:a) Microbial biomass, b) basal respiration rates and c) metabolic quotients (qCO2) of microbial communities from the L (black bars) and FH (grey bars) layer forest floors. Bars represent mean values (n = 3) of the studied stands dominated by mature temperate tree species (Oak, Beech and Radiata pine) and mechanized 16ys old radiata pine forest. Error bars represent one standard deviation. Values followed by different lowercase letters differ significantly for the effect of Species and values followed by different uppercase letters differ significantly for the effect of Management.

Mineralizable C or cumulative respired C was lowest in L layer beneath the oak stand, whereas beech and pine 40ys showed similar and slightly more mineralizable C than oak stand (Fig 3.3). Mature pine and beech stands showed very similar respiration patterns in the FH layer. Pine 16ys contained more readily-mineralizable C than the mature pine stand in both layers studied (L-layer; t= 8.17, $P < 0.05$ and FH-layer; t= 5.12, $P < 0.1$). As found with microbial biomass and basal respiration, mineralizable C decreased consistently with forest floor depth (Fig 3.3).

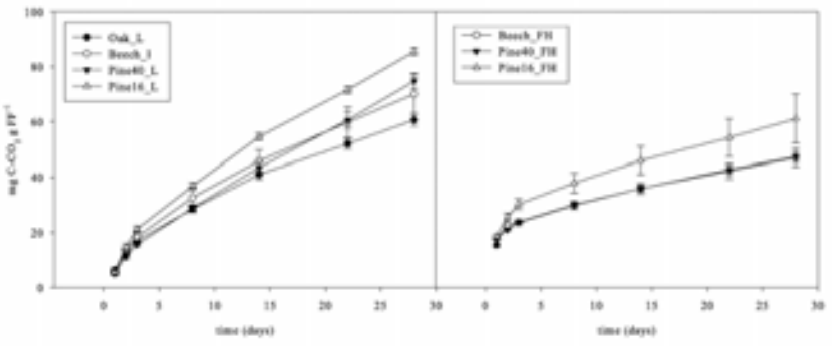

Figure 3.3: Cumulative respiration of microbial community over 28 days of incubation from a) L and b) FH layers. Values represent means ± SE (n = 3).

3.3.4. Diversity of forest floor microbial community

The number of substrate used (NUS) in L layer forest floor differed significantly depending on the species; oak and beech stands used an average of 17 and 18 substrates in 72 hours respectively from 31 substrates in the Biolog Ecoplates™, while pine 40ys used an average of 11, and pine

16ys an average of 12 substrates. In FH layers, the mean NUS used in beech FH layer was 15, whereas in pine 40ys and pine 16ys, mean NUS were 11 and 10 respectively. In terms of forest layer effects, microbial communities from L and FH layers did not differ significantly in NUS (data not shown).

The Shannon diversity index was significantly higher in oak and beech than in pine stand in L layer forest floor (Fig 3.4). In FH layer, microbial communities diversity index was similar in both mature stands and significantly lower in pine 16ys than Pine 40ys. The only significant difference in Shannon diversity between the two forest floor layers was observed in pine 16ys stand, for which the microbial diversity index was significantly lower in the lower forest floor layer.

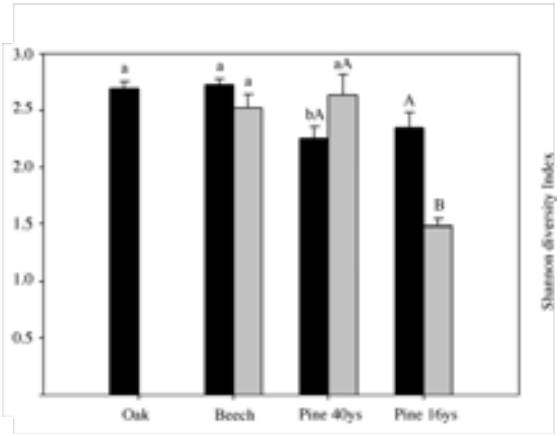

Figure 3.4: Shannon diversity index as a practical measure of the overall microbial functional diversity from the L (black bars) and FH (grey bars) layer of forest floors. Bars represent mean values (n = 3) of the studied stands dominated by mature temperate tree species (Oak, Beech and Radiata pine) and mechanized 16ys old radiata pine forest. Error bars represent one standard deviation. Values followed by different lowercase letters differ significantly for the effect of Species and values followed by different uppercase letters differ significantly for the effect of Management.

Canonical variate analysis of the PCA factors (Table 3.2) performed with the CLPP data discriminated microbial communities from L layers beneath different tree species on CV1 (F= 29.72, $P < 0.05$), with communities from beech trees having substantially higher coordinate values on this axis than oak, pine 40ys and pine 16ys. Although two forest floor layers were not discriminated in either canonical variate axis, the average distance between forest floor layers for the same tree species was 3.7 while the average distance between tree species from the same forest floor layer was 3.0 (Fig 3.5). Analysis of individual loadings of individual carbon sources (Table 3.2) on CV1 indicated that differences in microbial utilisation of benzoic acid, malic acid, phenylethylamine, ∝-cyclodextrin and glycogen were mainly responsible for division of the communities towards the positive side of this axis. D-galacturonic acid was mainly responsible

for the division of the communities towards the negative side this axis. N-acetyl-D-glucosamine was the main factor involved in the discrimination on CV2.

Analysis of variance of the utilisation of 6 different groups of carbon sources (amides, amino acids, carbohydrates, carboxylic acids, phenolic acids and polymerics) in the CLPP showed that microbial communities from L layer used more carboxylic acids (F= 12.2, $P < 0.01$) than those from FH layer; the converse being the case for polymeric carbon signals (F= 5.5, $P < 0.05$).

Table 3.2: Principal Component Analysis for reduction in dimensionality of all CLPP data. The first 10 PCA factors were retained for posterior application of the Canonical Variate Analysis (CVA). Factors marked in bold type accouned for the discrimination on CV1 and underlined factorsaccounted for the discrimination on CV2.

	Eigenvalue	% of Variance	Cumulative %
Factor 1	6.74	21.76	21.76
Factor 2	4.46	14.37	36.13
Factor 3	3.73	12.02	48.15
Factor 4	2.52	8.12	56.26
Factor 5	2.22	7.15	63.41
Factor 6	2.05	6.60	70.01
Factor 7	1.88	6.07	76.09
Factor 8	1.30	4.21	80.29
Factor 9	1.14	3.67	83.96
Factor 10	0.96	3.09	87.04

	Factor 1	**Factor 2**	Factor 3	Factor 4	Factor 5	**Factor 6**	Factor 7	Factor 8	**Factor 9**	Factor 10
Phenylethylamine						0.658				
Putrescine										
L-arginine										
L-asparagine	0.913									
L-Phenylalanine				0.794						
L-serine										
L-Threonine										
Glycyl-L-Glutamic Acid	-0.61									
C-Cellobiose					0.848					
D-Lactose		0.653								
Methyl-D-Glucoside										
D-Xylose										0.87
i-Erythritol							0.879			
D-Mannitol	-0.82									
N-Acetyl-D-glucosamine			-0.89							
D-Glucosaminic acid								0.802		
D-Galactonic acid lactone			-0.77							
D-Galacturonic acid									-0.788	
2-Hydroxy benzoic acid		0.826								
4-Hydroxy benzoic acid	0.797									
Hydroxy butyric Acid										
Traconic acid										
Keto butyric acid										
D-Malic acid		0.838								
Pyrubic Acid Methyl Ester		-0.71								
Glucose-1-Phosphate				0.732						
D,L-Glycerol Phosphate										
Tween 40			0.629							
Tween 80										
∝-cyclodextrin						0.854				
Glycogen						0.807				

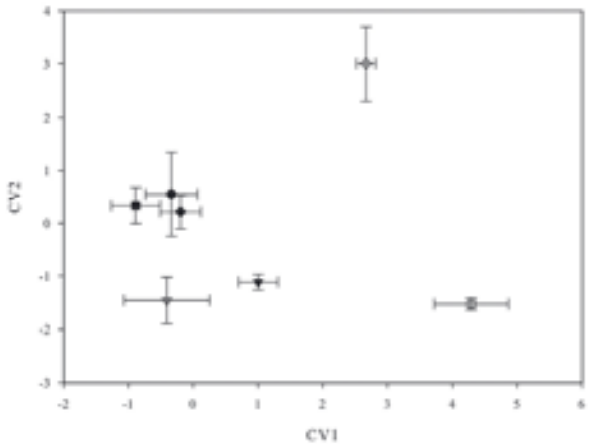

Figure 3.5: Discrimation of microbial communities from the forest floors based on CLPP profiles. Open symbols represent L layer samples and closed symbols FH samples. (●) oak, (■) beech, (▲) pine 40ys and (◆) pine 16ys. Values represent means ± SE (n = 3).

3.3.5. Relationship between carbon mineralization rates, litter quality and microbial community

Principal Component Analysis of all forest floor properties is shown in Table 3.6. Factor 1, which accounted for almost the half of the total variance in PCA showed that chemical properties of forest floor such as concentrations of Mg, K and Na were negatively correlated with biochemical properties such as ADF, CEL and lignin, and positively with canonical variate function 1 (CV1). These correlations may reflect the influence of chemical environment on microbial community functioning. Furthermore, microbial biomass and respiration rates were related to litter quality parameters (C/N, C/P and lignin/N ratios).

Factor 2 showed the relationship between forest floor moisture content, nitrogen, phosphorous and sulphur concentrations, primordial nutrients for microbial growth. Factor 3 reflected a negative relationship between Shannon diversity and the metabolic quotient (qCO_2) of microbial community. These results suggest that a more diverse microbial community may be more efficient in microbial C use.

Finally, forest floor mass, which may be indicative of *in situ* litter decomposition, forest floor pH, which may influence microbial community composition, and WSC, which may be the most labile C source, were not correlated with any of the forest floor properties studied.

Table 3.3: Principal Component Analysis of the selected forest floor properties.

Factor	Eigenvalue	% of Variance	Cumulative %
1	11.39	43.82	43.82
2	5.52	21.23	65.04
3	3.40	13.09	78.13

	Factor 1	Factor 2	Factor 3
mass (Mg ha^{-1})			
moisture (%)		0.886	
pH			
C (mg g^{-1})	0.951		
N (mg g^{-1})		0.762	
P (mg g^{-1})		0.843	
S (mg g^{-1})		0.824	
Ca (mg g^{-1})			
Mg (mg g^{-1})	-0.81		
K (mg g^{-1})	-0.916		
Na (mg g^{-1})	-0.854		
ADF (%)	0.934		
CEL (%)	0.92		
Lignin (%)	0.921		
WSC (mg g^{-1})			
MBC (mg C g^{-1})	0.883		
BR (mg C-CO_2 g^{-1} h^{-1})	0.854		
qCO_2			-0.797
Cmin (mg C-CO_2 g^{-1})	0.842		
CV1	-0.62		
CV2			-0.785
Shannon diversity Index			0.776
C/N	0.729		
C/P	0.745		
lignin/cel		0.761	
lignin/N	0.806		

3.4. Discussion

3.4.1. Effect of tree species on forest floor dynamics

The results indicate differences in detritus mass and consequently, in decomposition rates in forest floors of the studied stands. Litter from oak trees was so amenable that it did not form an FH layer. Differences in litter decomposition may be due to differences in litter characteristics or due to differences in soil animal activity. Raulund-Rasmussen and Vejre (1995) also found very fast forest floor decay rate under common oak in Danish forests and concluded that it was probably due to the effects of earthworms. On the other hand, beech stands showed higher forest floor accumulation than pine, which may be due to the greater amount of litterfall produced by beech

(Kavvadias et al., 2001 and Romanya and Vallejo, 2004). This is consistent with the findings of Kavvadias et al. (2001), i.e. higher forest floor accumulation in *Fagus sylvatica* than in *Pinus pinaster* and *Pinus nigra*, but faster litter decomposition rates in beech than in pine forests. The decomposition rate of forest floor beneath pine was lowest showing that 70% of the forest floor was located in FH layer.

Forest floor L layer under deciduous trees had a different chemical composition from that of pine forest floor. Forest floor pH, Ca and Mg under two deciduous trees (oak and beech) were higher than under coniferous trees. In organic matter with high concentrations of nutrients, bacterial activity dominates and nutrients are rapidly released (Melillo et al., 1982). However, comparison of microbial biomass of L layers did not reveal any differences between broadleaved and coniferous tree species. On the other hand, tree species can influence microbial decomposition primarily via differences in litter lignin (Hobbie et al., 2006). Litter with higher lignin/N and C/N ratios decomposes more slowly than litter with lower lignin/N and C/N ratios (e.g. Melillo et al., 1982; Prescott, 1995; Hobbie et al., 2006). Deciduous trees showed significantly lower C/N and lignin/N ratios than pine trees. However, there were significant differences between the two deciduous trees in terms of forest floor decomposition rates, which may be explained by differences in the potential utilisation of carbon sources by microbial communities (CLPP).

Microbial communities beneath beech species were clearly discriminated by CLPP. Forest floor beneath beech trees showed higher moisture content than forest floor beneath oak trees. As all samples were collected at the same time, these differences could be attributed to higher water storage capacity of the beech forest floor than oak forest floor due, for instance, to higher accumulation of organic matter. The difference in moisture content may influence the bacterial:fungal ratio of the microbial community. Grayston and Prescott (2005) also concluded that the high bacterial:fungal ratio in cedar forest floors in British Columbia may be related to their higher moisture content. Gram-negative bacteria, including pseudomonas, are particularly sensitive to water stress, and Gram-positive bacteria, particularly actinobacteria, are highly tolerant to water stress (Killham, 1994). Therefore, the composition of the microbial community may differ in forest floors beneath oak and beech trees, which may explain the differences in organic matter dynamics in the forest floor.

3.4.2. Effect of management in pine forests

The evidence for forest floor loss following logging operations was convincing and consistent with the results of Johnson et al. (1995) who found a significant decline in forest floor mass from 3 to 8 years after whole-tree harvesting. However, some studies in which the same stands were compared

before and after logging showed that forest floor mass increases when the aboveground organic residues are left in the stand (Mattson and Swank, 1989). The results of the present study reflect the total removal of the forest floor mass from the pine 3ys stand with the front blade of a bulldozer, and that this was still absent even 3 years after the establishment of the new pine plantation. Moreover, forest floor mass was still 50% lower compared with a mature stand 16 years after disturbance. Covington's curve (1981) predicted a loss of 50% of forest floor organic matter in the first 20 years after the disturbance due to a reduction in litter input and acceleration in decomposition rates after clear-cutting. In the corresponding study, a series of stands of different ages were used to describe the pattern of forest floor mass and organic matter content during succession following logging in northern hardwood stands in New Hampshire (Covington, 1981).

Clear-cutting and subsequent site preparation operations may also alter the quality of organic matter by increasing decomposition rates. Ussiri and Johnson (2007) found relatively small changes in the structure and composition of organic matter by C^{13} NMR after clear-cutting, however they concluded that more labile fractions and more dynamic pools of organic matter may be more sensitive indicators of the effects of disturbance. Water-soluble carbohydrates (WSC) and cumulative respiration increased significantly relative to the adjacent mature pine forest in the studied stand 16 years after clear cutting in the FH layer. However, we could not find any relationship between WSC and the forest floor properties studied. This may reflect higher decomposition rates even 16 years after disturbance (Covington, 1981).

Forest floor microbial community was also affected by clear-cutting and subsequent site preparation. The Shannon diversity index was significantly lower than that observed in mature pine and CLPP was also different in pine 16ys. Hannam et al. (2006) found that the forest floor microbial community responded immediately after harvesting but returned to pre-harvest levels in 4.5 and 5.5 years post-harvest for PLFA. In contrast, we suggest that the microbial community in the cultivated pine forest under study is not resilient following clear-cutting and subsequent site preparation during the first 16 years after disturbance.

3.5. Conclusions

Organic matter dynamics in the forest floor was different beneath different tree species. In the stands under study, oak, beech and pine forests showed different patterns of accumulation of organic matter. Although no differences were detected in microbial biomass, there were differences in litter quality and microbial diversity.

Mechanization of forest management in the *Pinus radiata* plantations under study induces complex changes in the forest floor. Results from this study indicate a significant loss of organic

matter following clear-cutting and mechanical site preparation, and that these changes may persist for at least 16 years after disturbance. In addition, accelerated decomposition rates were observed and substantially lower microbial diversity in the pine 16ys stand. Nevertheless, further studies are required to elucidate the effects of change in tree species and clear-cutting and site preparation on carbon dynamics and long-term site productivity at the landscape level.

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4. Potential indicators of soil quality for temperate forest ecosystems: A case study in the Basque Country

As intensification of forest management may affect the quality of the soil, the assessment of sustainable forest management requires reliable soil quality indicators. In this study, the responses of several chemical, physical and biological soil related parameters were evaluated under different types of forest management to know their usefulness as indicators. To do so, five adjacent forest stands were studied. On one hand, two seminatural broadleaved forests (Quercus robur, Fagus sylvatica) and an adult radiata pine plantation (40-year-old,) in order to study the effect of species change, and, on the other, a chronosequence of Pinus radiata plantations (40-year-old; 16-year-old, and 3-year-old), to study the effect of mechanization during harvesting and site preparation. Results revealed that measuring the responses of the selected parameters on top soils (0-5cm) is more effective to get sensitive indicators for both forest management types than measuring them in the subsoil (5-15cm). Chemical parameters did not appear as a sensitive indicator for species change, and only organic matter related chemical parameters (organic carbon, total nitrogen and phosphorus) indicated the disturbance caused by mechanical site preparation. Biological parameters proved sensitive indicators for both studied management impact; the fungal phospholipid fatty acid biomarker 18:2ω6 was sensitive to show tree species change and the ratio of Gram+/- bacteria was sensitive as an indicator of mechanization. Extracellular enzyme activities were sensitive to organic matter removal with mechanization. They might also indicate nutritional stress related to phosphorous deficiency. The least limiting water range (LLWR) was a very sensitive indicator of physical degradation induced by mechanical site preparation.

As a characteristic of good indicators is how they relate to a benchmark or target that define a sustainable level, further studies are needed in these forest ecosystems to assess natural boundaries of variation and the resilience of the selected soil properties.

4.1. Introduction

Most European countries agreed to the Pan-European principles for sustainable forest management (Helsinki Process, 1993) because conversion of native forests and afforestation of agricultural or abandoned land to provide cultivated forests of rapidly growing exotic species was a widespread phenomenon. Furthermore, the increasing concurrence in global markets and the intensification of logging operations (timber harvesting and site preparation) were placing greater pressure on forest ecosystems. In northern Spain, cultivated radiata pine (*Pinus radiata* D. Don) forests cover an area of more than 150,000 ha, and seminatural forests of European beech and pedunculate oak have been maintained in small patches (20,000 ha). The current types of forest management in these cultivated plantations involves clearcutting with rotations of between 30 and 40 years, harvesting with chainsaws, skidding, and mechanical site preparation, prior to planting, by processes such as scarification and ripping.

Forests play a key role in global sustainability not only as providers of raw materials, but also as providers of ecosystem functions related to biodiversity, carbon sequestration and protection of soil and water. Maintenance of these functions in perpetuity should be the goal of sustainable forest management (Doran, 2002). Assessment of the sustainability of forest management requires indicators, and as forestry has a large impact on soil systems, there is a need to develop soil-based sustainability indicators; this need has increasingly been identified since forest policies have shifted to more balanced ecological approaches. The main challenge in selecting soil quality indicators is determining which measures appropriately characterize the system and yet are simple enough to be effectively and efficiently monitored (Dale et al., 2007).

Several authors have proposed minimum datasets of soil parameters to be used as soil quality indicators (Doran and Parking 1994, Tscherko and Kandeler 1999, Shukla et al., 2006). These datasets need to define ecosystem processes that integrate physical, chemical and biological properties. Soil organic matter (SOM) has frequently been suggested as a key attribute of soil quality and sustainability because it is linked to nutrient availability, water holding capacity, C cycling, gas exchange and biological processes (Nambiar, 1996). However, results of prior studies in cultivated forests were inconclusive in terms of the response of total SOM as an indicator of sustainable forest management (Bauhus et al., 2002). Therefore, different pools of SOM with different functional roles in soil must be evaluated. Particulate organic matter (POM) may respond more rapidly to management than SOM (Cambardella and Elliot, 1992; Fortuna et al., 2003) and soil microbial community, a small but very active part of the SOM (Christensen, 1992) has been suggested as a sensitive indicator of change in grassland and boreal ecosystems (Mariani et al., 2006; Tscherko et al. 2007).

Microbial community composition and function may be altered by changes in the quantity and biochemical composition of plant litter (Hassett and Zak, 2005) and the effect of logging operations on soil microenvironments. Phospholipid fatty acid (PLFA) has been used to study the variability in forest soil communities (Leckie et al., 2004). Certain fatty acids are used as biomarkers for particular groups of organisms (Frostegard and Bååth, 1996) and total PLFA content as a measure of microbial biomass (Zelles et al., 1999). Microbial extracellular enzymes degrade e.g. the cellulose, hemi- cellulose and lignin of plant litter (Waldrop et al., 2003), and reflect the functioning of soil microbial communities and ecosystem processes.

Bulk density, soil strength, aggregate stability or available water content (AWC) have often been used as indicators of soil physical quality, because compaction caused by logging disrupts the physical integrity of soil by modifying the porosity and impeding movement of gas, water, nutrients and roots in the profile (Greacen and Sands, 1980). The least limiting water range (LLWR) has recently been suggested as a better indicator of soil physical disturbance as it incorporates the limiting values for the growth of the vegetation such as soil mechanical impedance, oxygen and water supply to plant roots into one parameter on the basis of water content. The lower limit is defined by the water content at which soil resistance to penetration becomes limiting which is considered 3 MPa for radiata pine (Zou et al., 2000) (wilting point or the threshold soil strength value), and the upper limit is defined by the water content at which aeration becomes limiting (field capacity or 10% air-filled porsity) (Zou et al., 2000). Intrinsic accountability of these important water limit criteria makes the LLWR a potentially useful soil quality indicator of mechanized forest system impact on soil physical conditions and vegetation growth (da Silva and Kay, 1996; da Silva and Kay, 1997b; Betz et al., 1998 and McKenzie and McBratney, 2001), however, rather few studies have been conducted in forest ecosystems.

Chemical parameters commonly used to evaluate soil quality, such as total nutrient content, change slowly over time, thus limiting their usefulness for detecting early changes induced by forest management (Bending et al., 2004). Although routine soil chemical analyses are not usually directly related to the capacity of the soil to supply nutrients in the long term, they are still the most common type of soil analysis applied both in agriculture and forestry and are widely use in management impact assessment.

The main characteristic of a good indicator of forest sustainability is its ability to be compared with a benchmark and/or target that defines a sustainable level. Natural forests have been identified as representing this sustainable level in terms of forest management. However, tree species affect soil processes such as nutrient cycling (Binkley, 1995), base content and humus

formation (Raulund-Rasmussen and Vejre, 1995). These effects are mainly attributed to the effect that species differences in litter quality and root exudates (Hobbie, 1992) have on soil microorganisms (Leckie et al., 2004). It is therefore important to understand the differences in soil properties with respect to native and exotic species in order to establish coherent sustainable forest benchmarks.

In this context, the objectives of this study were i) to understand the behaviour of a set of parameters that have been proposed as soil quality indicators in adjacent stands that differ mainly in land-use history and management but not in soil parent material, geomorphology or climate and ii) to obtain an insight into the potential use of these parameters as indicators of soil quality, for future planning of soil monitoring programmes in temperate forest ecosystems.

4.2. Materials and Methods

4.2.1. Site description

The study site (5 ha) (30T 534075 4783284) was selected as an example of the Atlantic forest landscape in the Basque Country. The Atlantic climate is mesothermic with cool, moist summers and mild, wet winters. The mean air temperature is 18.7 ºC in summer and 9.6 ºC in winter. The mean annual precipitation ranges between 1200 and 2000 mm and heaviest rainfall occurs between September and May (Ortubai, 1995). The area is situated 600 m above sea level. The parent material is sandstone and soil mineralogy and textural classes were homogeneous within the study site (Table 4.1). The soil was classified as a Typic Udorthent (SSS, 2006). The land mainly comprises steep hills to moderately steep rolling hills. Five different and adjacent forest stands were selected, three of them differing in tree species (native or exotic) and a chronosequence of exotic species plantations differing in time since site preparation: (1) Seminatural oak forest dominated by *Quercus robur* L. with *Erica vagans* L., *Ulex europaeus* Schott, *Rubus ulmifolius* L. and *Ilex aquifolium* L. in the understory and not managed in the last few decades, and (2) Seminatural beech forest characterised by *Fagus sylvatica* L. pollarded until the mid 1940s for firewood and charcoal and not managed in the last few decades. In addition, three *Pinus radiata* plantations that differ in age and silvicultural history were chosen, namely (3) Pine (40-year-old), a mature even-aged plantation established around 40 years ago without mechanical site preparation, and in which there is a sparse understory with ferns, and (4) Pine (16-year-old) and (5) Pine (3-year-old old), which are even-aged second-rotation plantations, established after logging. Logging involves three operations: timber harvesting, preparation of soil and ripping of log landing. Harvesting in both cases involved chainsaw felling and mechanical snigging of logs with a skidder, to a landing. Soil preparation involved the removal of logging

slash and surface organic material from the stand and scarification of surface soils with a front-mounted straight-blade; log landing was performed with a ripper tooth that digs into the soil and a plough that displaces the soil laterally and partially turns it over, creating a trench.

4.2.2. Soil sampling

Each stand covered an area of approximately 1 ha, and three study plots (10 m x10 m) were placed in each stand. Within each plot, ten soil samples were systematically collected by the core method (core diameter 15 cm) in January of 2005, and the samples were divided into 0-5 cm and 5-15 cm mineral soil layers. Soil samples were combined to provide one composite sample per plot and layer and were then split into a field-moist portion and a portion for drying. The field-moist portion was sieved to < 2 mm to remove large woody debris and stones, separated into two replicate samples and frozen at -20ºC. The other portion was dried at 30ºC and also sieved to 2 < mm and separated into two replicate samples. Subsamples were dried at 105 ºC for 24 h for estimation of the moisture content.

4.2.3. Chemical properties

Total organic C and N were measured in a LECO CNS 2000 autoanalyser (LECO Corporation, MI, USA). Soil pH-H_2O was determined in 1:2.5 soil:water suspension. Cation exchange capacity (CEC) was determined with ammonium acetate (1M, pH 7). Extractable aluminium was measured by titration with sodium hydroxide and phenolphthalein. Available phosphorus was analysed by the Olsen method (Olsen et al., 1954).

Particulate organic matter (POM) was determined by the method of Cambardella and Elliot (1992), as modified by Marriott and Wander (2006). Briefly, 10 grams of soil were dispersed with hexa-metaphosphate in an end-over-end shaker at 150 rpm for 16 hours, and the sample was then passed through a 53 μm sieved and rinsed with distilled water until a clear solution was obtained. The mixture of sand and POM was oven dried at 50ºC for 48 hours and the C content was determined with a LECO CNS 2000 analyser.

Table 4.1: Soil mineralogical and textural properties (all expressed in %) from 0-5 cm and 5-15 cm of the soil profile of seminatural oak and beech stands, and cultivated pine 40ys, 16ys and 3ys stands.

	Oak	Beech	Pine (40ys)	Pine (16ys)	Pine (3ys)
0-5 cm					
Mineralogy					
Quartz	47	48	42	49	47
Phyllosillicates	52	51	56	51	53
Clay mineralogy					
Illite	30	29	34	44	33
Chlorite	4	2	5	5	4
Kaolinite	16	14	9	8	14
I/V	51	55	52	42	48
Texture					
Clay	33	29	38	28	32
Silt	39	40	40	37	30
Sand	27	32	22	35	38
	clay loam	clay loam	clay loam	clay loam	clay loam
5-15 cm					
Mineralogy					
Quartz	43	46	43	49	50
Phyllosillicates	55	51	54	48	49
Clay mineralogy					
Illite	20	28	21	28	25
Chlorite	2	t	1	2	t
Kaolinite	16	13	13	10	13
I/V	62	60	64	60	61
Texture					
Clay	43	37	43	30	34
Silt	36	36	38	33	30
Sand	20	27	20	36	36
	clay	clay loam	clay	clay loam	clay loam

t = trace
I/V = illite/vermiculite

4.2.4. Biological properties

The microbial community structure was determined by phospholipid fatty acid (PLFA) analysis. Lipids were extracted with single phase, citrate buffered $CHCl_3$-CH_3OH solution (Bligh and Dyer, 1959; White et al., 1979). Phospholipid fatty acids were fractionated by silicic acid chromatography (Varian, CA, USA), and derivatized in an alkali system to form fatty acid methyl esters. Fatty acid methyl esters were analysed with a Perkin Elmer XL Autosystem (Perkin Elmer, CT, USA) equipped with a 50 m long column (0.2 mm i.d., 0.33 μm film thickness). The column temperature was held at 60 ºC for the first 2 min, increased to 160 ºC at 30 ºC per min, increased again to 280 ºC at 3 ºC per min and then held at this temperature for 15 min. The injector and detector were maintained at 260 and 280 ºC, respectively. Identification of peaks was based on the retention time and mass spectral comparison with known standards. The abundance of individual fatty acid methyl esters was expressed per unit dry weight. The nomenclature used for phospholipid fatty acids was that of Frostegard et al. (1993). Bacterial biomass was estimated

from the summed concentration of 9 bacterial PLFA, i15:0, a15:0, 15:0, i16:0, i17:0, 17:0, cy17:0, 18:1ω7 and cy19:0 (Frostegard and Bååth, 1996) and the fatty acid 18:2ω6 was used as an indicator of fungal biomass (Federle, 1986). The Gram-positive specific fatty acids i15:0, a15:0, i16:0 and i17:0 and the Gram-negative specific fatty acids cy17:0, 18:1ω7 and cy19:0 were used as biomarkers. Total phospholipid fatty acids were compared among different types of forest management as nmol g^{-1}soil, while PLFAs used as biomarkers were compared on a mole basis (%) in order to standardize differences in the total amount of soil PLFAs. The diversity of PLFAs was calculated with the Shannon index *H*.

The enzyme assay was performed as microbial community function by a modification of the method of Marx et al. (2001). Briefly, the enzyme activities assayed were: β-1,4-glucosidase, α-1,4-glucosidase, cellobiohydrolase, β-1,4-xylosidase, β-1,4-*N*-acetylglucosaminidase, acid phosphatase, L-leucine aminopeptidase and L-tyrosine aminopeptidase. One gram of fresh soil and 50 ml of sterile water were sonicated for 2 min at an output energy of 50 J s^{-1}. The resulting soil suspension was continuously stirred and 50 µl aliquots were dispensed in 96-well microplates. Fifty microlitres of buffer solution were added; glycosidases and acid phosphatase were assayed in 100 mM MES buffer (pH 6.1) and peptidases in 50 mM Trizma buffer (pH 7.8). Finally, 100 µl of 1 mM substrate solution was added. Depending on the substrate tested, either a 4-methylumbelliferone or 7-amino-4-methyl coumarin standard was used. The microplates were pre-incubated in the dark at 30ºC and the fluorescence intensity was then measured at time 0, 30, 60, 120 180 min in a Microplate Fluorescence Reader FLx800 (BIO-TEK instruments Inc., VE, USA) equipped with an excitation filter of 355 nm and an emission filter of 460 nm. All enzyme activities were expressed in nmol g^{-1} h^{-1}.

4.2.5. Physical properties

The least limiting water range (LLWR) was calculated in all stands on the basis of experimental data. Critical values for plant growth associated with water potential, soil resistance and air-filled porosity were selected: 0.10 cm^3cm^{-3} air-filled porosity (Grable and Siemer, 1968), field capacity at -0.01 MPa matric potential (Haise et al., 1955), wilting point at -1.5 MPa matric potential (Richards and Weaver, 1944), and the soil strength restriction limit at 3.0 MPa (Sands et al., 1979). Field capacity (θ_{fc}) and wilting point (θ_{wp}) water contents were determined in all samples by the standard tension plate procedure (Klute, 1986). The soil water content identified by the air-filled porosity at 10% (θ_{afp}) was calculated as θ_{sat}-0.1, where θ_{sat}= 1-(bulk density/soil particle density). Bulk density was estimated in ten soil samples extracted with stainless steel (100 cm^3) cylinders in each plot. Water content at 3 MPa (θ_{ss}) was determined on soil strength regression

models developed for each stand from a grid of 34 points. In each point, soil strength was measured with a cone penetrometer (CP 40II, Rimik, Australia), readings were recorded every 1 cm and the average value of the first 5 readings represented the soil strength in the first 5 cm. In addition, a soil sample was taken to determine the gravimetric water content. Sampling was carried out twice, once when soils were near field capacity (May 2005) and the second time when they were near wilting point (August 2005). The upper limit of the LLWR is the drier soil water content of either θ_{fc} or θ_{afp}, whereas the lower limit is the wetter soil water content of either θ_{wp} or θ_{ss} (Zou et al., 2000).

4.2.6. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to compare mean values for each soil depth and a Student t test to compare the two depths. Factor analyses were performed to reduce the number of variables in extracellular multienzyme activities and PLFA patterns with a correlation matrix; factors with eigenvalues >1 were retained and factors subjected to varimax rotation to maximize correlation between factors and measured properties. Stepwise discriminant function analysis was used to determine the key soil properties that best predict the forest categories and how good the prediction was. It was used to gain insights into the relationship between group membership and the variables used to predict group membership. When using any stepwise method, it is often desirable to determine the efficiency of the analysis via a cross-validation procedure to develop an unbiased estimate for determining to which group a particular observation belongs.

Once the discriminant functions were calculated, a plot of the stretched attribute vectors was calculated by rotating the discriminant functions to redistribute the variance. Correlations between the canonical discriminant functions and each discriminating variable were calculated and multiplied by the univariate *F* ratio to produce (*x, y*) coordinates for the endpoint of a vector that has its other endpoint at the origin (Hair et al., 1995). Thus, the *F* value indicates the extent to which each variable makes a significant contribution to discriminating between groups.

4.3. Results

4.3.1. Chemical soil properties

Soil organic C contents in the upper soil horizon were similar in all stands except the pine (3-year-old) (Table 4.2), concentrations of nitrogen, available phosphorus and potassium, and cation exchange capacity (CEC) were also significantly lower in the latter stand. There was a significant decrease with depth in all elements associated with soil organic matter, such as soil total nitrogen,

soil phosphorus and CEC. In contrast, soil pH increased with depth and was significantly higher in oak stands, but was almost always below 5 (Table 4.2).

However, although the quantity of soil organic matter did not differ among mature stands, the quality of the soil organic matter differed significantly with tree species. The contents of labile C and particulate organic matter (POM) were higher in oak and beech stands than in pine stands (40 years old). Conversely, a reduction in labile C with depth was observed in all stands except in the mature pine stand (Table 4.3).

Table 4.2: Soil chemical properties from 0-5 cm and 5-15 cm of the soil profile of seminatural oak and beech stands, and cultivated pine 40ys, 16ys and 3ys stands. Values in the table are the means of three replicates and values in parentheses represent standard errors.

	Oak	Beech	Pine (40ys)	Pine (16ys)	Pine (3ys)
0-5 cm					
pH	5.0 (0.2)^{a}	4.0 (0.1)^{b}	4.1 (0.1)^{b}	4.3 (0)^{b}	4.2 (0)^{b}
Al Sat (%)	29.3 (19.5)^{a}	81.7 (1)^{b}	83 (3.9)^{b}	71.7 (3.5)^{b}	78.3 (1)^{b}
C (mg kg^{-1})	54.5 (0.9)^{a}	53 (9.4)^{a}	53.8 (1.2)^{a}	56.8 (6.2)^{a}	26.8 (1.6)^{b}
N (mg kg^{-1})	3.7 (0.2)^{a}	3.1 (0.6)^{a}	3.1 (0.2)^{a}	2.9 (0.2)^{a}	1.8 (0)^{b}
C/N	14.9 (0.6)^{a}	17 (0.3)^{ab}	17.5 (1.4)^{ab}	19.1 (0.8)^{b}	15.1 (0.8)^{a}
S (mg kg^{-1})	0.5 (0.1)^{a}	0.6 (0.1)^{a}	0.7 (0)^{a}	0.5 (0)^{a}	0.4 (0)^{a}
P (mg kg^{-1})	3.1 (0.4)^{a}	3.3 (0.7)^{a}	0.9 (0.3)^{b}	2.9 (0.6)^{a}	0.3 (0.1)^{b}
Ca (mg kg^{-1})	739 (393)^{a}	71 (6)^{ab}	171 (48)^{ab}	179 (39)^{ab}	44 (8)^{b}
Mg (mg kg^{-1})	113 (33)^{a}	18 (2)^{b}	50 (10)^{b}	34 (7)^{b}	11 (3)^{b}
Na (mg kg^{-1})	38 (2)^{a}	31 (2)^{a}	38 (2)^{a}	37 (3)^{a}	20 (2)^{b}
K (mg kg^{-1})	208 (17)^{a}	110 (8)^{b}	104 (10)^{b}	120 (16)^{b}	52 (7)^{c}
CEC	21.1 (1.2)^{a}	19.2 (1.9)^{a}	25.8 (1.3)^{b}	18 (0.7)^{a}	11.3 (0.2)^{c}
5-15 cm					
pH	4.9(0.1)^{a}	4.6 (0)^{ab}	4.5 (0.1)^{b}	4.5 (0.1)^{b}	4.5 (0)^{b}
Al Sat (%)	44.7 (17.1)^{a}	91.7 (1.1)^{b}	90.3 (1.4)^{b}	87.3 (1.4)^{b}	84.3 (2.3)^{b}
C (mg kg^{-1})	22.1 (1.6)^{a}	19 (1.7)^{a}	25.7 (2)^{a}	19.5 (2.5)^{a}	20.3 (0.3)^{a}
N (mg kg^{-1})	1.7 (0.1)^{a}	1.2 (0.1)^{b}	1.6 (0)^{a}	1.2 (0.2)^{b}	1.3 (0.1)^{b}
C/N	13.3 (0.9)^{a}	15.9 (0.6)^{a}	16.2 (1.4)^{a}	16.7 (1.1)^{a}	15.6 (0.6)^{a}
S (mg kg^{-1})	0.3 (0)^{a}	0.3 (0)^{ab}	0.3 (0)^{a}	0.2 (0)^{b}	0.3 (0)^{a}
P (mg kg^{-1})	0.1 (0)^{a}	0.6 (0.2)^{ab}	0.4 (0.2)^{a}	1.2 (0.3)^{b}	0 (0)^{a}
Ca (mg kg^{-1})	876 (361)^{a}	27 (3)^{b}	78 (17)^{b}	69 (14)^{b}	21 (1)^{b}
Mg (mg kg^{-1})	83 (29)^{a}	8 (0)^{b}	26 (2)^{b}	16 (3)^{b}	6 (1)^{b}
Na (mg kg^{-1})	28 (3)^{a}	22 (1)^{b}	31 (1)^{a}	20 (1)^{b}	17 (1)^{b}
K (mg kg^{-1})	101 (3)^{a}	61 (4)^{c}	74 (10)^{abc}	77 (14)^{ac}	41 (4)^{bc}
CEC	17.7 (1.1)^{a}	12.5 (0.3)^{b}	19.2 (1.2)^{a}	11.6 (0.8)^{b}	11.3 (0.5)^{b}

Different letters in the same row represent significant difference at $P < 0.05$

4.3.2.Biological soil properties

a) Microbial community structure

Microbial biomass, considered as total amounts of PLFA, differed with intensive preparation of pine stands. The microbial biomass was similar in mature stands, of either native or exotic species. In contrast, microbial biomass was highest in the 16 year old pine stand and lowest in the 3-year-old pine stand (Table 4.3).

Soil microbial community was dominated by bacterial biomass, which accounted for 52-56 % of the total PLFA and as in microbial biomass, the only significant differences observed were between the 16 year old and 3 year old pine stands. In contrast, there were differences among tree species in fungal biomass; fungal biomass content was significantly higher in pine (40-year-old) than in oak and beech stands. Furthermore, fungal biomass was significantly lower in recently established stands than older pine stands (Table 4.3).

A shift in bacterial community was detected on the basis of PLFA biomarkers. Gram-positive bacteria (i15:0, a15:0, i16:0 and i17:0) made up an increasing component of the bacterial community in the upper horizon of pine (3 years old) ($P < 0.05$), while Gram-negative bacteria (16:1ω7, 18:1ω7, cy17:0 and cy19:0) were less abundant ($P < 0.05$) (Table 4.3). The ratio between Gram-positive bacteria and Gram-negative bacteria was significantly higher in the 3 year old pine stand ($P < 0.0001$) than in the other stands.

Microbial biomass in 16- and 3-year-old pine stands was significantly reduced in the lower soil layer, in comparison with the surface layer, but microbial biomass did not differ with depth in mature stands. Soil microbial community at depth was also dominated by 18:1ω7, 18:1ω9c and 16:0. The ratio between Gram-positive bacteria and Gram-negative bacteria increased with depth in all stands except the 3 year old pine stand (Table 4.3). There was also a clear reduction in the fungal to bacterial PLFA ratio from the surface to the deeper soil horizon ($P < 0.05$) (data not shown).

The PLFA diversity and species richness, as estimated by the Shannon diversity index, were similar in oak and beech stands and lower than in pine stands at 0-5 cm soil depth ($P < 0.05$) (Table 4.3).

Table 4.3: Particulate organic matter (mg C g^{-1} soil), microbial, bacterial and fungal biomass (nmol PLFA g^{-1} soil), Gram-negative and Gram-positive biomarkers (%mole PLFA) and Shannon diversity index H from 0-5 cm and 5-15 cm of the soil profile of seminatural oak and beech stands, and cultivated pine 40ys, 16ys and 3ys stands.

	Oak		Beech		Pine (40ys)		Pine (16ys)		Pine (3ys)	
0-5 cm										
POM	6.52	(0.27)^{a}	6.43	(1.58)^{a}	3.25	(0.25)^{b}	5.40	(0.01)^{ab}	1.63	(0.15)^{b}
Microbial biomass	210.59	(28.84)^{a}	221.12	(10.64)^{a}	239.21	(23.72)^{ac}	304.06	(26.09)^{c}	136.51	(4.0)^{b}
Bacterial biomass	115.67	(16.12)^{ab}	117.43	(3.83)^{ab}	125.02	(14.11)^{ab}	159.29	(13.43)^{a}	80.64	(1.08)^{b}
Gram+	23.95	(0.37)^{a}	25.47	(0.99)^{a}	23.59	(0.74)^{a}	23.00	(0.39)^{a}	34.49	(1.3)^{b}
Gram-	29.43	(0.93)^{a}	25.79	(0.34)^{b}	26.44	(1.58)^{ab}	27.75	(0.58)^{ab}	22.64	(0.35)^{bc}
G+/G-	0.82	(0.04)^{a}	0.99	(0.03)^{b}	0.90	(0.04)^{ab}	0.83	(0.03)^{ab}	1.53	(0.08)^{c}
fungal	6.63	(1.0)^{a*}	7.97	(1.21)^{ac}	12.85	(2.53)^{b}	12.05	(1.13)^{bc}	3.17	(0.84)^{a}
Shannon diversity *H*	2.50	(0.03)^{a}	2.58	(0.02)^{b}	2.65	(0.02)^{b}	2.65	(0.02)^{b}	2.61	(0.01)^{b}
5-15 cm										
POM	1.64	(0.05)^{a}	1.43	(0.13)^{a}	3.24	(0.51)^{b}	1.95	(0.48)^{a}	1.54	(0.11)^{a}
Microbial biomass	168.34	(6.81)^{ab}	147.03	(20.08)^{ab}	185.51	(20.56)^{a}	203.71	(27.75)^{a}	90.13	(4.52)^{b}
Bacterial biomass	89.90	(3.36)^{a}	82.11	(9.11)^{ab}	99.86	(7.09)^{a}	111.20	(14.47)^{a}	50.62	(2.86)^{b}
Gram+	27.08	(0.16)^{ab}	28.08	(0.76)^{ab}	25.68	(1.8)^{a}	24.41	(1.2)^{a}	30.91	(0.83)^{b}
Gram-	24.74	(0.67)^{a}	26.16	(1.12)^{ab}	26.71	(0.72)^{ab}	28.65	(0.91)^{b}	23.13	(0.44)^{ac}
G+/G-	1.10	(0.02)^{a}	1.08	(0.04)^{a}	0.96	(0.04)^{ab}	0.86	(0.06)^{b}	1.34	(0.06)^{c}
fungal	5.56	(0.84)^{a}	4.57	(0.83)^{a}	8.74	(3.47)^{a}	5.41	(0.42)^{a}	2.99	(0.44)^{ac}
Shannon diversity *H*	2.55	(0.05)^{a}	2.64	(0.02)^{ab}	2.60	(0.01)^{ab}	2.66	(0.02)^{ab}	2.71	(0.04)^{b}

* Significance level at $P < 0.1$

Values in the table are the means of three replicates and values in parentheses represent standard errors. Different letters in the same row represent significant difference at $P < 0.05$

b) Microbial community function

Extracellular enzyme activities involved in C-, N-, P- and S-cycling decreased within the soil profile in all sites (Table 4.4). The oak ecosystem was characterised by higher β-glucosidase and α-glucosidase activity in the upper soil layer (0-5 cm) than in the other sites ($P < 0.05$). Although the differences between most individual extracellular enzyme activities were not statistically significant, there was a 32-74 % reduction in all enzyme activities in the 3-year-old pine plantation in comparison with the average activity across other ecosystems.

Phosphatase activity was extremely high in all ecosystems in comparison with the values reported in previous studies (Larson et al., 2002; Sinsabaugh et al., 2003; Hasset and Zak, 2005). Mean phosphatase activity for the oak ecosystem was 7.5 μmol h^{-1} g^{-1}, for beech forest, 7.1 μmol h^{-1} g^{-1} and for pine (40-year-old), pine (16-year-old) and pine (3-year-old), 8.1, 5.7 and 3.5 μmol h^{-1} g^{-1} respectively. The acid phosphatase activity was significantly higher ($P < 0.1$) in mature stands than in the other stands.

Data obtained from the upper soil by a multi-enzyme assay with MUB substrates were used for principal component analyses and the initial eight enzyme variables were reduced to three factors. PCA1 explained 50 % of the variance and was associated with polysaccharide decomposition and strongly correlated with the activities of three enzymes involved in the production of glycosides and cellobiohydrolase. PCA2 explained 20 % of the variance and was associated with the N cycle (amino peptidases). PCA3 explained 18 % of the variance and was related to mineralization of organic phosphorus. Xylosidase activity, which causes degradation of hemicellulose, was correlated with PCA3. At the lower soil depth, PCA1 and PCA2 explained 48% and 26% of the total variance respectively. PCA1 was associated with C and P cycles and PCA2 with N cycle, however it was not possible to separate the different types of forest management.

4.3.3. Physical soil properties

Soil moisture content at sampling declined significantly from 0-5 cm to 5-15 cm depth in all stands except the pine stand (3-year-old), in which there was no difference in moisture level between the two horizons and the values were very close to those observed in the deeper horizon in the other stands. Bulk densities from 0-5 cm depth ranged from 1.09 g/cm^3 in pine (40-year-old) to 1.35 g/cm^3 in the pine (3-year-old) stand and soil water content at field capacity decreased with increasing bulk density (Table 4.5).

Soil water content at field capacity ranged from 0.24 cm^3/cm^3 in the pine stand (3-year-old) to 0.40 cm^3/cm^3 in oak stand, and water contents at field capacity were higher in all mature stands

than in younger stands (3 and 16-year old). In addition, soil water content at wilting point ranged from 0.10 cm^3/cm^3 to 0.20 cm^3/cm^3 and was also lowest in the 3-year-old pine stand (Table 4.5).

The relationship between soil strength and water content was established in each plot over the range between near field capacity and wilting point. Soil strength increased as soil water content decreased in all plots. Soil strength curves were fitted to a logarithmic model (Zou et al., 2000) (Fig. 4.1). The highest estimate of *a* corresponded to the oak stand, whereas the estimates for the beech, and the 40-year-old and 16-year-old pine stands were very similar and the lowest estimate was again of the 3-year old pine stand.

However, water content at 3 MPas was higher in all mature stands than in younger stands.

Table 4.4: Extracellular enzyme activities in nmol g $soil^{-1}$ h^{-1} from 0-5 cm and 5-15 cm of the soil profile of seminatural oak and beech stands, and cultivated pine 40ys, 16ys and 3ys stands.

	Oak	Beech	Pine (40ys)	Pine (16ys)	Pine (3ys)
0-5 cm					
ß-1,4-glucosidase	446.3 (17.6)^{a}	271.6 (51.9)^{b}	256.6 (30.4)^{b}	264.1 (47.4)^{b}	148.9(25.5)^{b}
a-1,4-glucosidase	363.1 (38.4)^{a}	192.4 (24.7)^{b}	236.1 (32.7)^{b}	206.3 (28.5)^{b}	129.2 (37.7)^{b}
ß-1,4-xylosidase	176.1 (34.9)^{a}	175.9 (32.6)^{a}	205.2 (15)^{a}	185 (36.8)^{a}	105.7 (27.5)^{a}
Cellobiohydrolase	49.3 (7.6)^{a*}	30.3 (11.3)^{ac}	23.3 (5.3)^{bc}	23.1 (3.3)^{bc}	11 (3.3)^{b}
ß-1,4-N-acetylglucosaminidase	408.6 (76.8)^{a}	281.5 (100.5)^{a}	267.4 (49.6)^{a}	299.3 (64.8)^{a}	217.9 (60.9)^{a}
L-leucine aminopeptidase	555.8 (27.5)^{a}	323.1 (58.3)^{a}	346.8 (118.1)^{a}	333.7 (22.2)^{a}	88.6 (14)^{a}
L-tyrosine aminopeptidase	237.6 (5.6)^{a}	142.7 (20.7)^{a}	149.3 (43)^{a}	159.5 (5)^{a}	53.7 (6.8)^{a}
Acid phosphatase	7516.3 (1375)^{a*}	7072.1 (1121)^{a}	8071.5 (974)^{a}	5740.1 (467)^{ab}	3492.8 (258)^{b}
5-15 cm					
ß-1,4-glucosidase	93.7 (26.8)^{a*}	37.8 (16.5)^{b}	93.9 (10.3)^{ab}	47 (7.8)^{ab}	34.9 (8.1)^{b}
a-1,4-glucosidase	74.9 (16.5)^{ab}	45.4 (6.7)^{bc}	96.3 (11.1)^{c}	35.3 (7)^{c}	33 (7)^{c}
ß-1,4-xylosidase	51 (8.8)^{ab}	49.7 (6.9)^{a}	70.2 (1)^{ab}	55 (11.2)^{ab}	39.6 (8.2)^{ab}
Cellobiohydrolase	13.5 (2.3)^{a}	8.7 (2.4)^{a}	6.1 (2.7)^{a}	2.7 (1.4)^{a}	8.2 (3.2)^{a}
ß-1,4-N-acetylglucosaminidase	138.4 (42.5)^{a*}	63.7 (13)^{ab}	162.8 (43.9)^{b}	88.3 (27.8)^{b}	84.3 (14.1)^{ab}
L-leucine aminopeptidase	173.7 (8.1)^{ab}	383.4 (242)^{a}	149.8 (16.7)^{a}	135.2 (29.3)^{a}	231.2 (154.1)^{a}
L-tyrosine aminopeptidase	79.4 (5.4)^{a}	240.7 (150.8)^{a}	100.6 (5.2)^{a}	88.7 (12.7)^{a}	165.5 (105.1)^{a}
Acid phosphatase	3443.4 (274)^{a}	2401.4 (269)^{a}	4589 (362)^{a}	2482.7 (226)^{a}	3263.1 (685)^{a}

***** Significance level at $P < 0.1$

Values in the table are the means of three replicates and values in parentheses represent standard errors. Different letters in the same row represent significant difference at $P < 0.05$.

Table 4.5: Critical points for determination of least limiting water range (LLWR) for root growth. ρ_b, bulk density (g/cm^3), θ_{fc}, water content at field capacity (-0.01 MPa), θ_{wp}, water content at wilting point (-1.5 MPa), θ_{ss}, water content at 3 MPa soil strength, θ_{afp}, water content at 10% air filled porosity and AWC, water available capacity. Values in italics represent the upper and lower limit of LLWR.

	Oak	Beech	Pine (40ys)	Pine (16ys)	Pine (3ys)
ρ_b	1.20	1.29	1.09	1.15	1.35
θ_{fc}	*0.40*	*0.36*	*0.39*	*0.31*	*0.24*
θ_{wp}	0.18	0.15	0.20	0.15	0.10
θ_{ss}	*0.22*	*0.20*	*0.22*	*0.17*	*0.19*
θ_{afp}	0.44	0.41	0.49	0.46	0.38
LLWR	0.19	0.16	0.18	0.14	0.05
AWC	0.28	0.21	0.19	0.16	0.14

The upper limiting factor of LLWR was always defined by water content at field capacity while the lower limit factor was characterized by water content at 3 MPa soil strength. The least limiting water range was as follows: oak > pine (40-year-old) > beech > pine (16-year-old) > pine (3-year-old) (Table 4.5). In all cases, LLWR was lower than the traditional available water capacity (AWC, the difference between field capacity and wilting point) showing that the available water for plant growth is smaller than the one determined with AWC. The LLWR was 22 and 34% lower than AWC in the oak and beech stands respectively, 10 and 16% lower in the 40-year-old and 16-year-old pine stands respectively and 300% lower in the 3-year-old pine stand. The results showed that a 15% increase in bulk density can lead to a 236% reduction in LLWR.

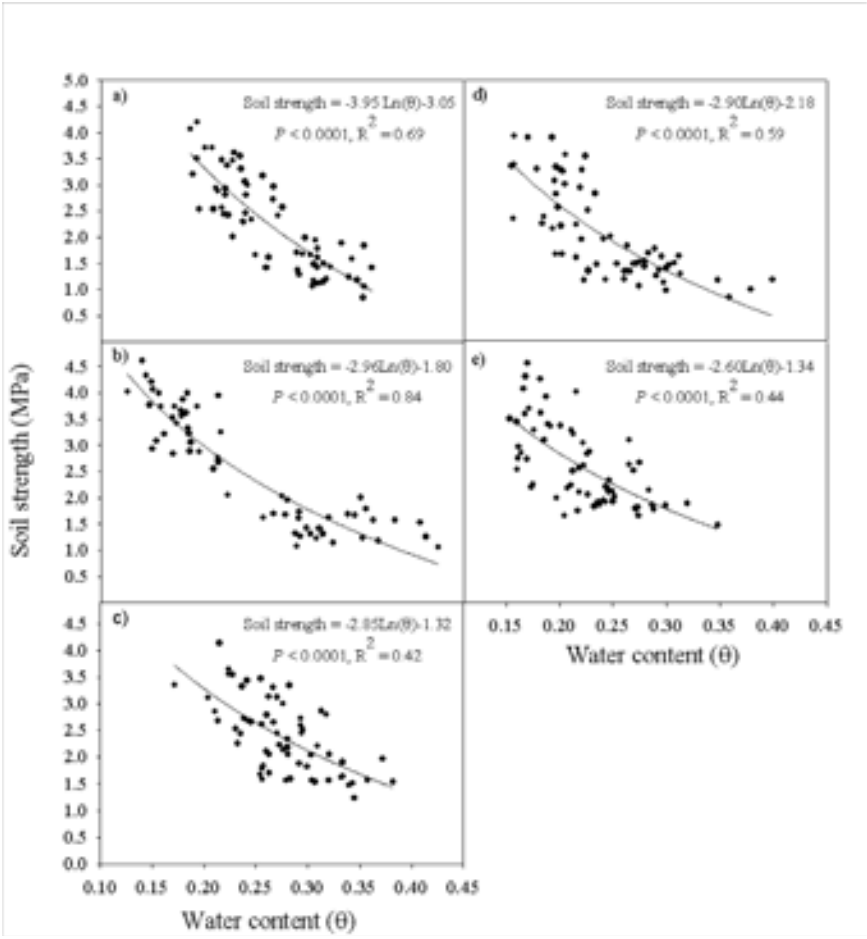

Figure 4.1: Soil strength regression models developed for each stand from data corresponding to a grid of 34 points in the range between near field capacity and wilting point in the 0-5 cm soil layer, a) oak, b) beech, c) pine (40-year-old), d) pine (16-year-old) and e) pine (3-year-old).

4.3.4. Identifying soil key properties

In order to differentiate among stands and determine which indicators were giving rise to site discrimination patterns, discriminant function analysis was used. Discriminant analysis predicts group membership based on a linear combination of potential variables. Discriminant analyses were performed on the basis of top soil chemical, physical and biological properties. The analysis considered a total of 29 potential indicators: 13 chemical, 12 biological and 4 physical. Although 94 % of the total variance was explained with chemical parameters alone (parameters shown in Table 4.1 + POM), only 53 % of cross-validated grouped cases were classified correctly (Fig 4.2). Physical parameters alone (bulk density, water contents at field capacity and wilting point and water contents at 10% air-filled porosity) were able to group 73 % of cross-validated grouped cases correctly. Discriminant analysis for biological parameters was based on microbial, bacterial and fungal biomass, Gram+, Gram- and Gram+/- ratio and the reduced factors from extracellular enzyme activities (3 PCA factors explaining 88 % of the total variance) and PLFAs (3 PCA

factors explaining 80% of the total variance). Biological parameters were able to group 53 % of cross-validated grouped cases correctly and the discrimination was due to PCA1 and PCA3 from multivariate analysis of enzyme activities, PCA3 from multivariate analysis of PLFAs and Gram+/- ratio. PCA1 and PCA3 from multivariate analysis of enzyme activities were related to enzymes involved in C and P cycles and PCA3 from multivariate analysis of individual PLFAs was related to fungal biomarkers.

The generated model based on a total of 29 properties was highly accurate, and correctly classified all stands, with 90 % of stands correctly classified in cross-validation. A classification error in cross-validation occurred among oak and mature pine stands, and a third of oak stands were classified as pine (40-year-old) and *vice versa*. This might reflect that the 40-year-old pine stand shares some characteristics with the oak stand.

The relationships between individual soil properties or discriminating variables and forests were revealed by the stretched attribute vectors (Fig 4.3). Of the 29 variables considered, 7 variables representing the greatest amount of information deal with soil physical, chemical and biological properties, which suggests that these properties may be useful in differentiating forests that differ in species and in mechanization intensity. Water contents at field capacity and 10 % air-filled porosity, parameters related to soil organic matter and compaction, were higher in mature stands than in mechanized younger stands. These changes could lead to differences in the structure of soil microbial community.

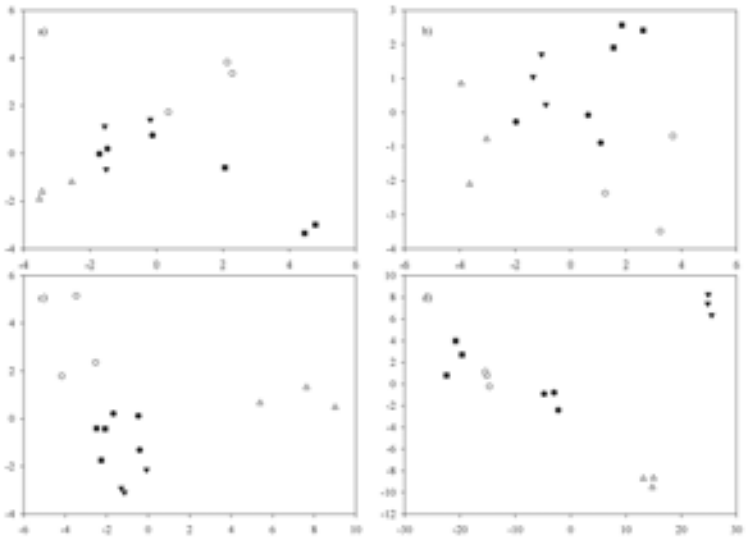

Figure 4.2: Discriminant analysis for the 0-5 cm soil layer, based on a) soil chemical parameters, b) physical parameters, c) biological parameters and d) soil chemical, physical and biological parameters. Stands are represented as follows: oak (o), beech (●), pine (40-year-old) (■), pine (16-year-old) (▼) and pine (3-year-old) (Δ).

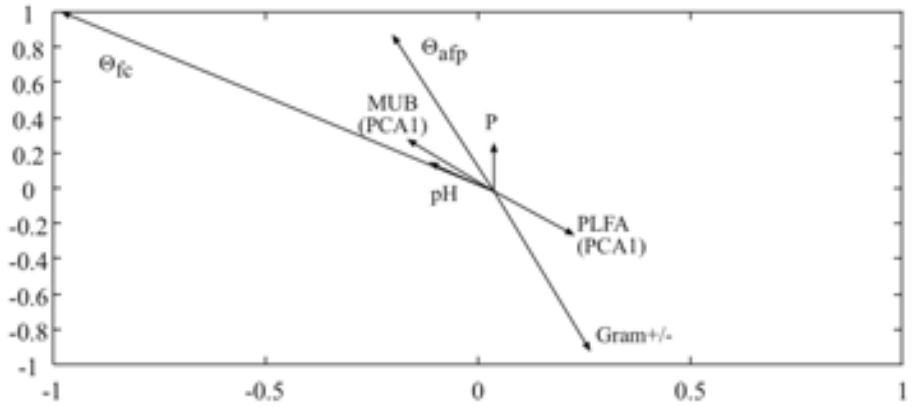

Figure 4.3: Attribute vectors plot calculated by rotating the discriminant functions of soil chemical, physical and biological parameters in the 0-5 cm soil layer.

4.4. Discussion

4.4.1. Depth effect on soil properties

Differences among species may be more likely to be caused by differences in the amount and quality of litter and by differences in processes taking place on the forest floor (Hagen-Thorn et al., 2004), as such, changes in all soil properties were more pronounced in the upper layer of mineral soil. Conversely, differences between logged (harvest, site preparation and ripping) and unlogged pine forests may be caused by changes in the quantity and quality of litter as well as alterations in the soil physical structure (Hassett and Zak, 2005). The lack of differences in the lower layer of the forest soils may be due to the similarity of soil organic matter contents in all stands and very slight differences in soil organic matter quality and consequently, similar biological activity of microbial community. An increase in abundance of Gram-positive bacterial PLFA and lower extracellular enzymes activities with depth (Yong-Mei et al., 2005; Niemi et al., 2005) may result from changes in water potential and a reduction in organic matter within the soil profile (Waldrop et al., 2003, Hasett and Zak, 2005). This pattern therefore has an important functional characteristic in nutrient cycling dynamics and implications for nutrient management in forest plantations.

4.4.2. Effect of tree species on soil properties

The results revealed that organic matter dynamics in pine forests and oak and beech forests may differ because of the higher fungal biomass and lower POM in pine forests. Hackl et al. (2005) also found higher concentrations of fungal fatty acids in pine forests than in beech or oak and

suggested that this may be because fungi are presumably more efficient than bacteria at decomposing pine litter. Pine litter contains high amounts of tannins and lignins and it is well known that fungi are the main organisms that cause lignin degradation (Dix and Webster, 1995). Part of the fungal biomass measured as fungal PLFA in pine forest soils may be derived from ectomycorrihzal fungi (Olsson, 1999), which are known to be dominant colonisers of the Pinaceae family (Smith and Read, 1997). Furthermore, Salas et al. (2003) found a correspondence between fungal colonization and POM-P, which indicated that the dynamics of this macroorganic matter may significantly influence P cycling and that the P contained in this OM pool may have a significant effect on P availability. It may be interesting to focus further studies on the dynamics of POM-P and fungal biomass, taking into account that because of the strongly acidic nature of the soils in northern Spain, the main factor limiting tree growth is the availability of P (Attiwill and Adams, 1993; Sanchez-Rodríguez et al., 2002). In the Basque Country, P-Olsen levels below 5 ppm and P-HCl levels below 150 ppm were found in 70 % of forests studied (Martínez de Arano, 2001), probably because phosphate is immobilized in Fe- and Al-complexes at low pH (Lajtha and Schlesinger, 1988). Such deficiencies could explain the very high phosphatase activities observed in the present study, related to the negative relationship between phosphatase activity and total P in different forests (Olander and Vitousek, 2000, Waldrop et al. 2003). Since phosphatase activity is related to soil organic matter (Tarafar and Jungk, 1988, Bonmati et al., 1991, Santruckova et al., 2004), the soil microbial community appears to invest a lot of energy in the production of this enzyme and in the aquisiton of inorganic P for microbial growth (Clarholm, 1993).

Bulk densities were significantly higher in oak and beech stands than in the mature pine stand. Forest soils may be compacted by grazing animals and by the roots of the trees themselves (Greacen and Sands, 1980) and the present results suggest that oak and beech roots become the soil more compacted than pine roots, however the differences in bulk densities were not reflected in water contents at field-capacity, wilting-point, 10 % air-filled porosity or soil strength at 3 Mpa. Furthermore, LLWR -defined as an index of soil quality for plant growth- was very similar in all mature stands and higher than the values reported by Leao et al. (2006) for native Cerrado forest in Brazil.

4.4.3. Logging effect on soil properties

The effects of soil organic matter removal and alteration in soil physical structure induced by logging were very pronounced in the youngest pine stand, at 3 years after establisment. Total C and some chemical parameters related to SOM such as cation exchange capacity (CEC) were

significantly lower in this stand than in the other stands. The observed decline in microbial biomass was consistent with the removal of organic matter (Hasset and Zak, 2005), and increased intensity of physical disturbance causes a decline in fungal biomass (Calderson et al., 2001, Gattinger et al., 2002). The bacterial community also shifted in the recently logged stand and there was an increase in abundance of Gram-positive bacterial PLFA with a concomitant decrease in Gram-negative bacterial PLFA. The present results are consistent with those of previous studies carried out in different ecosystems (Whyte et al., 2002, Margesin et al., 2003, Margesin et al., 2007), and can be explained by differences in microbial populations in terms of patterns of growth and reproduction. Gram-positive soil bacteria are able to use more complex carbon sources, such as mature SOM, and survive in resource limited ecosystems (*K* strategist); however, Gram-negative bacteria rely on easily degradable recent plant carbon sources and grow under substrate-rich conditions (*r* strategists) (Kramer and Gleixner, 2008). Dale et al. (2007) also found higher Gram-positive PLFA indicators in pine forests with moderate- and heavy-intensity military use in Georgia, USA, and Siira-Pietikainen et al. (2001) also concluded that the PLFA pattern was a sensitive indicator for identifying stress conditions induced by harvesting and mechanical site preparation in microbial communities, resulting from harvesting in spruce forests in Finland.

Soil physical properties were significantly altered by logging operations even at 16 years after plantation establishment. Compaction produced by vehicles used in the forest operations during establishment was still evident in the 16 year-old pine stand in comparison with the mature unlogged pine stand. Changes in soil structure are reflected in water content and gas diffusion, and therefore in plant growth (da Silva and Kay, 2004). As expected, water content at 10 % air-filled porosity declined with increasing bulk density, and water content at field capacity also decreased with decreasing soil organic matter.

The results obtained show that LLWR was more effective than AWC at reflecting changes in soil strength, pore size distribution and water retention in a meaningful single parameter. Similar values of LLWR to those obtained for pine (3-year-old) were reported for grazed pasture (Leao et al., 2006) and even lower values in soils under wheel tracks (Chan et al., 2006). Soils with a narrow LLWR are vulnerable to the effects of both drought and heavy rainfall (da Silva and Kay, 1997), and Benjamin et al. (2003) found that narrow LLWR can reduce the potential of a soil to support plant growth. The least limiting water range may therefore be a sensitive indicator of soil quality for plant growth.

4.4.4. Identifying soil key properties

The ocurrence of singularities and multi-colinearities that are present when all the indicators are used in the analysis were solved by use of the stepwise discriminant method (Stevens, 2002). Furthermore, when groups are defined *a priori* (as in this study), stepwise discriminant analysis is better than another commonly used method to describe multivariate data, namely Principal Component Analysis (PCA) (Tabachnik and Fidell, 2001). The 7 soil properties selected by discriminant function analysis represent the range of ecological indicator types that were surveyed in the present study: soil chemical, physical and biological properties. Water content at field capacity was consistently correlated with soil organic matter, and water content at 10 % air-filled porosity with bulk density. The stretched attribute vector analysis revealed that these physical parameters correspond to mature stands, which reflects the influence of logging operations on both soil organic matter and soil physical structure. A shift in soil microbial community was observed in the recently logged pine stand, and thus the ratio of Gram+/- bacteria appears to be a promising microbial indicator for detecting changes caused by forest management. The presence of phosphorus in discriminant function analysis reflects the nutritional stress in these forest ecosystems. The results suggest that an adequate minimum dataset for evaluating the sustainability of forest management should reflect this diversity.

4.5. Conclusions

Development of more sustainable forestry practices and credible certification systems relies on continuous monitoring of indicators. The National Forest Inventory (NFI), which is the only monitoring programme existing in Spain, provides information on the development of aboveground forest resources. However, assessment of the sustainability of forest management requires soil-based sustainability indicators as forestry largely affects soil systems. The conclusions reached in the present study could be used as a starting point for planning soil monitoring programmes.

Since the values of many soil properties, especially soil biological properties, decreased within soil profile, the upper 5 cm of the mineral horizon may be recommended as the most appropriate for evaluating changes due to forest management. Discriminant function analysis revealed that a diverse set of ecological indicators may be needed to assess the impact of forest management on soil quality. Parameters such as the ratio of Gram+/- bacteria, water content at field capacity and concentration of phosphorus may be responsive to logging operations. LLWR may be effective at reflecting changes in soil strength, pore size ditribution and water retention in a meaningful single

parameter. PLFA fingerprints (i.e. fungal biomarker) were also able to detect more subtle shifts in soil function, probably due to changes in litter quality and quantity.

All of these properties may be valuable and complementary tools that provide insights into soil ecosystem processes. However a characteristic of a good indicator of forest sustainability is how it relates to a benchmark and/or target that defines a sustainable level, and further studies are needed in temperate forest ecosystems to assess the boundaries for sustainability and the resilience of these soil properties.

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5. Soil organic matter in soil physical fractions in adjacent seminatural and cultivated forests in the Basque Country

Changes from natural tree species to rapidly growing exotic species as well as intensification of forestry operations can lead to changes in the quantity and quality of organic matter inputs to soil and disruption of soil physical structure, the two ecosystem properties that are most probably linked to organic matter dynamics. Five adjacent forest stands were selected to study soil organic matter dynamics in soil physical fractions. Two seminatural broadleaved forests (Quercus robur, Fagus sylvatica) and an adult radiata pine plantation (40-year-old,) in order to study the effect of species change, and, a chronosequence of Pinus radiata plantations (40-year-old; 16-year-old, and 3-year-old), to study the effect of mechanization during harvesting and site preparation. Samples of intact topsoil (0-5 cm) were collected and aggregate size distribution, mean weight diameter (MWD), total C and N, particulate organic matter (POM)-C, POM-N and microbial biomass were determined in each aggregate size fraction. Microbial respiration and nitrogen mineralization were also assessed in each aggregate size fraction, during a 28 day incubation period.

Losses of POM-C and POM-N in the whole soil due to mechanical site preparation were high relative to total soil C and N, which suggests that POM is sensitive to the effect of mechanization. The proportion of C-POM:SOM was significantly related to MWD ($R^2 = 0.75$, $P < 0.001$). Semi-natural forests were able to be distinguished from cultivated and from recently mechanized forests by the proportion of macroaggregates (0.25-2 mm). Megaaggregates (> 2 mm) were more abundant in mature stands (82-92 %), whereas macro- and microaggregates (< 2 mm) were more abundant in mechanically prepared Pinus radiata pine plantations (49 %).

The concentrations of C and N in the soil under study were not higher in larger aggregates, which indicates that POM may play a key role in macroaggregate formation.

Soil aggregate distribution and POM may be useful indicators for decision-making regarding the most appropriate management practices for increasing soil sustainability.

5.1. Introduction

Conversion of native forests and afforestation of agricultural or abandoned land into cultivated forests of rapidly growing exotic species is a worldwide phenomenon induced by increasing population growth and demand for wood and fibres. In addition, the increasing concurrence in global markets is putting greater pressure on forest ecosystems, which is usually accompanied by intensification of harvesting and site preparation practices. Cultivated radiata pine (*Pinus radiata* D. Don) forests cover more than $4x10^6$ hectares in the southern hemisphere (mainly in Chile, New Zealand, Australia and South Africa) but only around 300,000 ha in the northern hemisphere, mostly in northern Spain (Lavery and Mead, 1998). Forests previously dominated by *Quercus robur* L., were mostly felled by the end of the XIX century and were reforested during the XXth century with radiata pines in the Basque Country. Dominant tree species affect the availability and biochemical composition of organic matter inputs to soil (Leckie et al., 2004) and different root systems also affect aggregation differently, in relation to different root properties, exudates and functions (Chan and Heenan, 1999b).

The current plantation management in cultivated radiata pine forests in the Basque Country involves a clear-cut regime with rotation lengths between 30 and 40 years, harvesting with saw chain and skidding, and mechanical site preparation prior to planting with processes such as blading and ripping. These forestry practices may alter the amount and turnover rates of soil organic matter (SOM). Harvesting and site preparation result in considerable disturbance to the forest floor and a consequent redistribution of organic matter across the site (Merino et al., 2004); ripping breaks soil aggregates and exposes the previously protected organic matter to microbial decomposition (Ashagrie et al., 2007). Apart from the detrimental effect of harvesting and site preparation on soil structure and soil quality (Horn et al., 2004), the released organic C may contribute to global warming (IPCC, 2000).

Maintenance or enhancement of SOM in forest ecosystems is crucial for sustainable use of forest resources because of the multiple effects of SOM on nutrient dynamics (Binkley, 1995), C cycle (Nambiar, 1996) and soil structure.

SOM improves soil structure by enhancing aggregation and therefore it influences soil water movement and retention, aeration, erosion, nutrient recycling, root penetration and production (Bronick and Lal, 2005). The role of physical structure in determining SOM sequestration and turnover has generated much interest in recent years (Six et al., 2004, Liao et al., 2006). The relationship between organic matter and soil aggregation was proposed by Tisdall and Oades (1982) in a conceptual model and these authors considered three different cementing or binding agents: temporary, transient and persistent. Persistent binding agents consist of degraded,

aromatic humic materials associated with polyvalent metals strongly sorbed to clays, responsible for the construction of microaggregates (0.053-0.250 mm). In contrast, temporary and transient binding agents such as polysaccharides, roots and hyphae are responsible for the stabilization of microaggregates into macroaggregates (> 0.25 mm). Alternatively, macroaggregates may form around particulate organic matter (POM). POM is composed of partially decomposed plant and animal residues (Christensen, 2001) and as POM is decomposed and microbial exudates are released, the macroaggregate becomes more stable, the C:N ratio decreases, and microaggregates form inside (Oades, 1984). Because of the relatively labile nature of temporary and transient binding agents, soil management has a greater effect on macroaggregates and the organic matter retained on them, than on microaggregates, which have more stabilized and humified organic matter (Six and Jastrow, 2002).

The effects of tree species on soil aggregation and of intensive forest practices on soil aggregate distribution may be of great importance in regulating accretion or depletion of SOM in temperate Atlantic forests. To gain insights into the interactions between SOM structure and dynamics in temperate forests, natural aggregates from the field should be studied (Pulleman and Marinissen, 2004).

Understanding these relationships in mature semi-natural and cultivated forests and in plantations differing in management intensity is of particular interest for developing sustainable forest management practices. Five adjacent forest stands with different species, and in which different silvicultural treatments had been applied, were selected as a case study. The main objective of this case study was to gain insight into the following: i) the distribution of standing stock of soil C and N pools in forest stands differing in tree species and in forest plantations with different silvicultural history, ii) the effect of forest management (species change and mechanization) on the temporary binding agents and its relationship with soil aggregation and iii) the role of soil aggregates in active C and N pools in response to forest management.

5.2. Materials and methods

5.2.1. Site description

The study site (30T, 534075, 4783284) was selected as an example of the landscape of the Atlantic forest landscape in the Basque Country. Climate in this region is mesothermic, characterized by cool, moist summers and mild, wet winters. The mean air temperature is 18.7 ºC in summer and 9.6 ºC in winter. The mean annual precipitation ranges between 1200 and 2000 mm (Ortubai, 1995) and

is distributed rather evenly through the year, although maximum levels are observed in autumn and in spring (González-Arias et al., 2006).

The study area is situated 600 m above sea level. Five different and adjacent forest systems situated on a Typic Udorthent (SSS, 2006) and under the same climatic conditions, were selected. Three mature stands were selected and the effects of tree species on soil aggregation and on soil organic carbon (SOC) pools were evaluated. The dominant species in the stand were oak (*Quercus robur* L.), beech (*Fagus sylvatica* L.) and pine (*Pinus radiata* D. Don):

(1) Oak: Semi-natural oak forest dominated by *Quercus robur* with *Erica vagans*, *Ulex europaeus*, *Rubus ulmifolius* and *Ilex aquifolium* in the understorey and not managed over the last century,

(2) Beech: Semi-natural beech forest characterized by *Fagus sylvatica* pollarded until mid 1940s for firewood and charcoal and not managed in the last few decades.

(3) Pine40ys: an even-aged mature plantation plantation of *Pinus radiata* established around 40 years ago. At that time, sites were usually established manually. The understorey of this stand consisted mainly of a sparse cover of ferns.

In addition, two younger pine stands adjacent to the above mentioned stands were selected, to evaluate the effect of mechanized forestry practices in commercial cultivated forests relative to the mature pine stand. These plantations were a 16 year old stand and a 3 year old stand; both were established with the mechanized techniques shown below:

(4) Pine16ys: a 16 year-old even-aged second-rotation forest harvested with chainsaw felling and mechanical snigging of logs to a landing with a skidder. Preparation of soil involved displacement of logging slash residues and surface organic material out of the stand with a front-mounted straight-blade; seedlings were planted after down-slope ripping.

(5) Pine3ys: A recently established 3 year-old pine plantation established after mechanized site preparation similar to the previous one.

5.2.2. Soil sampling

Soil samples were collected from the first 5 cm of the mineral soil, by the pit method, in April of 2005. Within each stand, three pits were dug and undisturbed soil samples were taken from the first 5 cm (≈ 2000 cm^3) after removing forest floor material. After sampling, large soil clods were broken gently by hand. Additional three 5 cm deep soil cores (ø: 5 cm) per stand were taken to

determine soil bulk density. A soil sample was taken adjacent to these pits, for whole soil analysis. The samples were air dried and sieved < 2mm prior to analysis.

5.2.3. Soil analysis

Samples of the whole soil were analysed for chemical and physical properties to ensure that forest stands did not differ substantially in these properties. Soil pH was determined in water (1:2.5), and cations (K, Ca, Mg) were determined after extraction with 1.0 M NH4-acetate. Phosphorus was extracted with sodium bicarbonate (0.5 M; pH 8.5) and determined by colorimetry, with an UV-Visible spectrophotometer Varian Cary 100 (Varian, Inc., Palo Alto, Ca.) (Watanabe and Olsen, 1965; AOAC, 1980). Cation exchange capacity (CEC) was determined after saturation of the sample with 1N sodium-acetate. The Na was then removed with a solution of NH4-acetate (1N) and Na was determined on a Varian Spectraa 220 FS (Varian, Inc., Palo Alto, Ca.) by flame atomic absorption photometry (AOAC, 1980). Extractable cations were also determined by atomic absorption photometry, with the above mentioned apparatus. Aluminium was extracted with a BaCl2 solution (0.6N) and determined by titration with sodium hydroxide and phenolphthalein (Mosquera, 1990).

Soil particle analysis was performed by laser diffractometry with a Mastersizer 2000 diffractometer (Malvern Instruments, Worcestershire, UK) and the undisturbed soil cores were oven dried at 105ºC for 24 h and weighed for determination of bulk density (Table 5.1).

Table 5.1: Soil physical and chemical properties from 0-5 cm depth of seminatural oak and beech forests, and cultivated pine forests (40-year-old, 16-year-old and 3-year-old).

	Oak	Beech	Pine 40ys	Pine 16ys	Pine 3ys	*p value*
Bulk density ($g\ cm^{-3}$)	1.20	1.29	1.09	1.15	1.35	
Texture class	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam	
pH	4.39	4.68	4.74	4.46	4.91	0.4
Sat Al %	85	68	74	78	84	0.52
P (ppm)	2.9	1.8	2.7	2.5	0.5	0.13
Ca (ppm)	103.0	416.0	208.0	182.7	128.7	0.25
Mg (ppm)	31.7	95.2	46.0	48.8	30.1	0.22
K (ppm)	118.0	152.5	114.7	151.0	51.0	0.09
CEC (meq/100g)	18.3	23.4	24.3	17.6	69.7	0.18

5.2.4. Aggregate-size distribution

Soil samples were allowed to dry at 4 ºC to minimize the impact of air-drying on soil microbial communities and activities (Schutter and Dick, 2002) until gravimetric water content of about 80 g H_2O kg^{-1} soil was reached, and they were then divided into four portions. The soil was fractionated into aggregates by a dry-sieving method because external mechanical stresses such as ripping are thought to be the main causes of aggregate breakdown in the studied ecosystems, rather than drying-rewetting cycles. Moreover, dry sieving of soil would disrupt the physical habitat of soil microbial communities to lesser degree than wet-sieving (Schutter and Dick, 2002) and some of the studied parameters are related to the microbial community and its function.

Fractionation was achieved by placing 100 g of each of the soil portions mentioned above on nested sieves mounted on Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany). Sieves were mechanically shaken (amplitude 1.5 mm) for 2 min to separate soil into the following aggregate size classes: > 20, 20-10, 10-5, 5-2 mm (large macroaggregates or megaaggregates),

2.0-0.25 (macroaggregates); 0.25-0.053 mm (microaggregates), and < 0.053 mm (silt+clay size fraction).

Fractionated samples were later combined to make composite samples for each aggregate size class. Mean weight diameter (MWD = Σ (percentage of sample weight on sieve x the mean diameter of the size classes) was calculated as an index that characterizes the structure of the whole soil (Six et al., 2000 b).

5.2.5. Soil Organic Matter and Particulate Organic Matter

Samples of whole soil and separated aggregates were air-dried and ground to pass a 250 μm sieve for total carbon and nitrogen analysis in a LECO CNS 2000 autoanalyzer (LECO Corporation, MI, USA).

Soil organic matter (SOM) was also fractionated in particulate organic matter (POM) in both whole soil and soil aggregates. POM was defined as the organic matter in soil of size greater than 53 μm, and was determined according to the method of Cambardella and Elliot (1992) and modified by Marriott and Wander (2006). Briefly, 10 grams of whole soil and soil aggregates were dispersed with sodium hexametaphosphate (50 g L^{-1}) in a reciprocal shaker at 180 rpm for 6 hours, after which the solution was passed through a 53 μm sieve and rinsed with distilled water until a clear solution was obtained. The mixture of sand and POM was dried in an oven at 50 ºC for 48 hours and the associated C and N determined in a LECO CNS 2000 analyser after the mixture was ground to pass a 250 μm sieve.

The sand content (> 53 μm) of whole soil and aggregate fractions was determined and all carbon and nitrogen contents were expressed as sand-free carbon or nitrogen (Six et al., 2002).

5.2.6. Mineralizable C and N

Mineralizable C and N were determined in the five macroaggregate classes, representing between 95 and 99 % of the whole soil, by use of an alkali absorption technique (incubation for 28 days at 30 ºC). Ten grams of each aggregate fraction (moistened with distilled water until two thirds of field capacity) were placed in 250 ml beakers and incubated in the dark. The CO_2 evolved was trapped in 10 ml 0.2 N NaOH and determined on days 1, 2, 3, 8, 14, 22 and 28 by titration with 0.1 N HCl to the phenolphthalein endpoint, after precipitation of carbonates with $BaCl_2$. Basal respiration rate (BR) was expressed as microbial respiration rate at the end of incubation period (μg $C\text{-}CO_2$ g $aggregate^{-1}$ h^{-1}). Inorganic soil N (NH_4^+ and NO_3^-) was determined at the commencement and end of the incubation after extraction of 10 g of aggregate with 50 ml 2M KCl and posterior analysis with a flow injection autoanalyser (Alpkem, MD, USA). Mineralizable N (N_{min}) was calculated as the difference between N contents before and after the incubation period and expressed as mg N_{min} kg $aggregate^{-1}$.

5.2.7. Microbial biomass carbon

Microbial biomass carbon was determined for the whole soil and for the three smallest macroaggregate sizes (5-10, 5-2, and 2.0-0.25 mm). It was determined by fumigation-extraction method (Vance et al., 1987). Ten grams of moist whole soil and aggregate fraction were fumigated with alcohol-free chloroform for 48 h in an evacuated dessicator. Fumigated and non-fumigated samples were extracted with 40 ml 0.5 M K_2SO_4 and shaken for 1 h on a reciprocal shaker. The extracts were filtered using Whatman nº 42 papers and maintained frozen at -20ºC until analysis. Extractable C was determined by oxidation with chromic acid and colorimetric determination. Microbial biomass carbon (MB_c) was calculated as the difference between fumigated and non-fumigated samples divided by K_2SO_4 extract efficiency factor for microbial C (k_c = 0.379, Vance et al., 1987).

5.2.8. Statistical analyses

Statistical analyses performed to show differences among stands and aggregate fractions within a stand were carried out with Statview 5.0 (SAS, 1998). Variables were tested for normality and Levene´s test for homogeneity of variances prior to one-way analysis of variance (ANOVA), and a protected least-significant difference (PLSD) test was used to determine the significance of the main effects determined by ANOVA. These statistical analyses were performed to gain a deeper

insight into the data structure. Nevertheless, because of the inherent large variability usually found in forest ecosystems and the lack of stand replicates, statistical analyses were used to show trends in change among the studied stands rather than to draw absolute conclusions about the effects of species change or mechanical operations on soil. As such, significant differences at $P< 0.10$ were also considered (Six et al., 2002).

Reduced major axis regressions between different organic matter fractions were used to gain an insight into the relationships. PAST software was used for this purpose (Hammer et al., 2001).

5.3. Results

5.3.1. Distribution of soil aggregate fractions

The aggregate fractions obtained by dry-sieving in mature stands (oak, beech and pine 40ys) consisted mainly of megaaggregates (82-92 %), mixed with relatively small amounts of macroaggregates and microaggregates. In contrast, a drastic decrease in megaaggregates and a simultaneous increase in macroaggregates + microaggregates were observed in the recently established pine 3ys stand (Table 5.2). Macro and microaggregates represented 48 % of the whole soil in pine 3ys. In pines 16ys and 40ys these aggregates made up 18-21 % of the total.

These differences in aggregate distribution also induced changes in mean weight diameter (MWD) for the effect of tree species ($F= 7.27$, $P < 0.05$) and time since perturbation ($F= 25.07$, $P < 0.0001$). Both seminatural stands (oak and beech) showed significantly higher MWD than pine 40ys stand. Among *Pinus radiata* plantations, the pine3 stand showed the significantly smallest MWD while pine 16ys showed similar MWD to that observed in mature pine stand (Table 5.2).

Table 5.2: Soil aggregate distribution in g aggregate kg^{-1} soil (standard deviation) after dry-sieving from 0-5 cm of the soil of seminatural oak and beech forests, and cultivated pine 40ys, 16ys and 3ys forests.

Aggregate size (mm)	Oak	Beech	Pine (40ys)	Pine (16ys)	Pine (3ys)
Silt+Clay					
<0.053	0.32 (0.04)	0.47 (0.03)	0.84 (0.57)	0.85 (0.42)	3.62 (3.24)
Microaggregates					
0.053-0.25	5.07 (2.46)	8.27 (0.78)	11.04 (9.57)	13.11 (4.85)	40.60 (16.30)
Macroaggregates					
0.25-2mm	99.87 (59.62)	75.79 (10.22)	169.16 (40.24)	194.08 (34.48)	447.73 (6.58)
Megaaggregates					
2-5mm	207.44 (57.07)	145.28 (45.02)	290.57 (32.88)	293.13 (34.30)	317.03 (31.15)
5-10mm	265.63 (33.73)	168.24 (28.71)	342.16 (5.32)	253.38 (19.71)	148.84 (10.64)
10-20mm	326.50 (109.81)	288.66 (24.70)	186.24 (20.25)	173.28 (9.52)	42.18 (8.00)
>20mm	95.17 (41.51)	313.29 (107.90)	0.00 (0.00)	72.17 (48.29)	0.00 (0.00)
Macro + microaggregates	104.94 (62.1)	84.06 (37.5)	180.20 (49.3)	207.18 (39.3)	488.33 (17.5)
Megaaggregates	894.75 (62.1)	915.46 (9.5)	818.96 (49.9)	791.96 (39.5)	508.05 (17.1)
MWD	9.53 (1.85)	12.45 (1.40)	6.57 (0.34)	7.19 (0.94)	3.36 (0.11)

5.3.2. Soil Organic Matter in soil physical fractions

Values of total carbon and nitrogen concentrations and C/N ratios in the whole soil of the studied stands (oak, beech, pine 40ys, pine 16ys and pine 3ys) did not differ significantly (Table 5.3). However, the distribution of organic carbon and nitrogen in soil aggregates (Table 5.3) differed in semi-natural (oak and beech) and the mature cultivated stand (pine 40ys). The concentration of carbon decreased from macro- to megaaggregates in oak (Organic C, F= 53.77, $P < 0.0001$ and N, F= 32.93, $P < 0.0001$) and beech stands (Organic C, F= 2.84, $P < 0.05$ and N, F= 2.57, $P < 0.1$), whereas the concentration of carbon was homogeneous across different aggregate sizes in pine 40ys. A similar result with a homogeneous C concentration in different aggregate classes was also found in pine 16ys, but the pine 3ys stand showed a significantly higher concentration of carbon in 10-20 mm megaaggregate size than in the lower sizes (F= 3.34, $P < 0.1$) (Table 5.3).

Major differences in soil aggregate C and N among the mature stands under study were found in the 2-5 mm size class. The concentrations of these elements in oak and pine 40ys were almost half the concentrations in beech. On the other hand, although there were no significant differences in

concentrations of C and N in the studied pine stands in any of the aggregate sizes, values of these parameters in pine 3ys were as much as half of the values in pine 40ys in the macroaggregate size class.

The ratios of C/N did not differ significantly in the whole soil and soil aggregates (Table 5.3) in the studied stands, which shows that this may be a very conservative characteristic that may not be affected by forest species or management intensity.

Table 5.3: Organic C and N concentrations, g kg^{-1} sand-free aggregate (standard deviation), and C/N ratios in whole soil and soil aggregates from 0-5 cm of the soil of seminatural oak and beech forests, and cultivated pine 40ys, 16ys and 3ys forests.

	Aggregate size (mm)	Oak	Beech	Pine (40ys)	Pine (16ys)	Pine (3ys)
C (g C kg^{-1} sand-free aggregate)	Whole soil	60.53 (14.94)	101.70 (1.45)	83.78 (34.79)	65.66 (19.15)	52.24 (16.81)
	<0.053	*	*	58.70 (39.10)	*	26.85 (1.98)
	0.053-0.25	68.75 (35.78)	77.43 (35.89)	56.33 (7.81)	43.20 (9.78)	24.95 (5.02)
	0.25-2	94.86 (4.34)	171.74 (93.71)	113.00 (30.52)	79.50 (11.86)	44.62 (7.89)
	2-5	53.44 (3.86)	101.66 (20.95)	67.33 (16.73)	50.07 (6.66)	44.94 (13.21)
	5-10	38.79 (5.25)	73.38 (13.62)	63.15 (16.60)	41.92 (9.51)	38.81 (8.88)
	10-20	33.33 (5.69)	53.21 (4.32)	70.53 (34.39)	51.03 (30.11)	68.60 (17.82)
	>20	23.35 (7.20)	62.82 (26.71)		63.91 (56.57)	
N (g N kg^{-1} sand-free aggregate)	Whole soil	3.58 (0.37)	6.00 (0.35)	4.87 (1.74)	3.80 (1.04)	3.01 (0.91)
	<0.053	*	*	3.82 (1.55)	*	1.79 (0.03)
	0.053-0.25	4.01 (1.35)	4.83 (2.27)	3.25 (0.42)	2.71 (0.25)	1.50 (0.25)
	0.25-2	5.27 (0.41)	9.85 (5.74)	6.38 (1.27)	4.36 (0.64)	2.68 (0.39)
	2-5	3.22 (0.45)	5.95 (1.37)	4.11 (0.67)	2.93 (0.53)	2.78 (0.66)
	5-10	2.28 (0.15)	4.35 (0.82)	3.94 (0.95)	2.42 (0.43)	2.38 (0.40)
	10-20	1.92 (0.24)	3.00 (0.22)	4.32 (1.81)	2.91 (1.52)	4.31 (1.58)
	>20	1.69 (0.45)	3.74 (1.76)		3.51 (2.67)	
C/N	Whole soil	16.79 (2.44)	16.99 (1.25)	16.95 (1.18)	17.25 (0.40)	17.34 (0.31)
	<0.053	*	*	14.47 (4.35)	*	14.97 (0.82)
	0.053-0.25	16.58 (3.36)	16.04 (0.10)	17.33 (0.17)	15.89 (2.71)	16.63 (0.56)
	0.25-2	18.08 (2.22)	17.68 (0.79)	17.56 (1.17)	18.24 (0.89)	16.60 (0.54)
	2-5	16.83 (3.55)	17.14 (0.43)	16.24 (1.46)	17.19 (0.95)	16.06 (0.92)
	5-10	17.15 (3.45)	16.87 (0.05)	15.96 (0.87)	17.24 (0.98)	16.23 (0.99)
	10-20	17.65 (5.14)	17.74 (0.13)	16.02 (1.33)	17.17 (1.12)	16.26 (1.81)
	>20	13.70 (0.64)	17.01 (0.87)		17.20 (2.34)	

* not enough sample for analysis

5.3.3. Particulate Organic Matter in soil physical fractions

The quantity of POM-C in the whole soil sample amounted to 17-22 % of the total organic carbon in mature stands (Table 5.4). This parameter was reduced to 14 % in pine 16ys and to 5 % of the total organic carbon in pine 3ys stand, which was significantly lower than that observed in mature pine (F= 12.54, $P < 0.05$). The percentage of POM-N from total N showed a similar pattern. The POM-N fraction amounted to 15 % of total N in oak stand and 13 % in beech and pine 40ys stand. POM-N fraction represented 8 % of the total N in pine 16ys stand and it was reduced significantly, to 2 %, in pine 3ys stand (F= 9.54, $P < 0.05$). The C/N ratio of POM fraction, which is usually considered as an indicator of the quality of this fraction, did not differ significantly among mature stands. On the other hand, this parameter increased significantly in the plantations in which soil disturbance took place (F= 45.89, $P < 0.001$) (Table 5.4), with the highest value in pine 3ys and an intermediate value in pine 16ys.

The importance of POM fraction in soil aggregation was reflected in the significant exponential relationship between MWD and mg POM-C g^{-1} C total ($R^2 = 0.68$, $P < 0.05$).

The concentrations of POM-C and POM-N were significantly higher in aggregates of 0.25-2 mm than in the rest of aggregate sizes in all mature stands (Oak, POM-C F= 7.07, P < 0.05 and POM-N F= 6.13, P < 0.05), (Beech, POM-C F= 3.45, P < 0.1 and POM-N F= 2.58, P < 0.1), (Pine 40ys, POM-C F= 3.30, P < 0.1 and POM-N F= 2.61, P = 0.1). Although the concentrations were lower in the pine 16ys stand, the trend was the same as in the mature stands, with the highest concentrations of POM-C and POM-N in the macroaggregates (POM-C F= 10.64, P < 0.01 and POM-N F= 8.24, P < 0.01). In contrast, a homogeneous distribution POM was observed across all aggregate sizes in pine 3ys stand (Table 5.4).

Oak, beech and mature pine stands did not differ significantly in terms of the C-POM and POM-N in any aggregate size class (Table 5.4). However, concentrations of POM-C and POM-N were higher in the pine 40ys stand than in younger and disturbed stands, in the macroaggregate size class (0.25-2 mm) (POM-C, F= 8.38, $P < 0.05$ and POM-N, F= 8.80, $P < 0.05$). Finally, as observed in whole soil samples, the C/N ratios of POM in each aggregate size class in pine 40ys differed significantly from the C/N ratio present in pine 16ys and pine 3ys (Table 5.4).

5.3.4. Mineralizable C and N and microbial biomass C in soil physical fractions

A high variance was encountered in all studied microbial parameters, mainly in microbial biomass carbon in soil physical fractions. This caused non-significant differences, despite huge differences in mean values (Table 5.5). Therefore, the methodological approach for determining microbial

biomass carbon in soil aggregates should be improved due to the difficulties related to working with undisturbed soil fractions, and more replicates are recommended. However, some statistically significant differences were found in mineralizable C and N.

Basal respiration rates were significantly higher in the macroaggregates (0.25-2 mm) than in the megaaggregates (> 2 mm) in all mature stands (Oak, F= 4.03, $P < 0.05$; Beech, F= 3.95, $P < 0.05$ and Pine 40ys, F= 4.36, $P < 0.05$), but there were no significant difference among tree species. Conversely, homogeneous C_{min} rates were observed across all aggregate sizes in the two soils under younger stands subjected to mechanical site preparation (pine 16ys and pine 3ys). Nevertheless, the intensive mechanization in the recently established plantation (pine 3ys) significantly reduced the mineralizable C relative to pine 40ys (F= 7.83, $P < 0.05$) (Table 5.5).

In a similar way, mineralizable N differed significantly among different aggregate size classes in semi-natural mature forests. Mineralizable N tended to be reciprocally related to the aggregate size in oak and beech stands (Oak, F= 4.57, $P < 0.05$ and Beech, F= 4.74, $P < 0.05$); however, in pine 40ys, a the value of N_{min} was homogeneous across all aggregate sizes. Similarly, there were no significant differences in N_{min} among aggregate sizes in younger and disturbed stands. Although the dynamics observed in all studied pine stands was similar, pine 3ys showed lower mineralization of N than pine 40ys in the macroaggregate size class and in the 5-10 mm size class. Pine16ys again showed an intermediate value, suggesting that mechanization may also reduce the amount of mineralizable N and showing a trend towards recovery with time, but that 16 years may not be sufficient time (Table 5.5).

The metabolic quotient (qCO_2) showed similar trends in all stands except the pine 3ys stand. The qCO_2 values were higher in aggregates of size 0.25-2 mm than in larger aggregates. This trend was significant in oak stand (F= 3.79, $P < 0.05$). On the other hand, qCO_2 values were lower in pine 3ys than in pine 40ys in all size classes, which shows that mechanization may tend to increase the efficiency in C use in all aggregate sizes (Table 5.5).

The microbial parameters studied were significantly related to soil organic matter fractions. Mineralizable C was significantly and positively related to POM-C (r = 0.85, $P < 0.0001$), and mineralizable N was also significantly (although less so) related to POM-N (r=0.37 $P < 0.01$) and basal respiration (r= 0.39 $P < 0.01$).

Table 5.4:Particulate organic matter C and N concentrations, mg C or POM-N g^{-1} sand-free aggregate (standard deviation), C/N ratios of POM and POM-C/SOC and POM-N/N total ratios in whole soil and soil aggregates from 0-5 cm of the soil of seminatural oak and beech forests and cultivated pine 40ys, 16ys and 3ys forests.

Stand	Aggregate size (mm)	C-POM	N-POM	C/N-POM	mg C-POM /g C	mg N-POM /g N
Oak	Whole soil	13.15 (1.96)	0.54 (0.05)	24.10 (1.33)	22.00 (2.19)	15.22 (0.14)
	0.25-2	27.48 (11.33)	1.18 (0.53)	23.57 (1.08)	29.28 (13.28)	22.03 (8.44)
	2-5	18.02 (5.37)	0.65 (0.23)	28.18 (1.77)	34.18 (12.51)	19.76 (4.41)
	5-10	7.21 (0.16)	0.26 (0.04)	28.00 (3.22)	18.78 (2.96)	11.37 (0.81)
	10-20	4.05 (0.41)	0.12 (0.03)	36.90 (14.11)	12.21 (0.85)	5.97 (0.99)
	> 20	2.61 (1.38)	0.10 (0.05)	27.29 (0.52)	10.79 (2.59)	5.43 (1.45)
Beech	Whole soil	18.05 (2.68)	0.76 (0.20)	24.11 (2.87)	17.76 (2.89)	12.60 (2.61)
	0.25-2	47.34 (24.20)	2.44 (1.51)	20.47 (2.64)	25.27 (4.59)	22.20 (5.97)
	2-5	27.66 (10.88)	1.40 (0.66)	20.54 (2.50)	22.32 (7.06)	18.95 (7.46)
	5-10	19.02 (7.21)	0.96 (0.45)	20.85 (3.04)	21.20 (5.15)	17.54 (5.90)
	10-20	13.05 (6.84)	0.61 (0.41)	23.05 (3.88)	18.56 (2.68)	14.39 (3.93)
	> 20	10.63 (1.32)	0.47 (0.00)	22.62 (2.82)	19.10 (10.22)	14.14 (6.66)
Pine 40ys	Whole soil	14.37 (7.17)	0.64 (0.38)	23.27 (2.28)	16.69 (1.64)	12.42 (3.34)
	0.25-2	31.30 (10.68)	1.43 (0.52)	21.92 (2.10)	27.79 (7.21)	22.13 (4.64)
	2-5	16.14 (10.42)	0.71 (0.52)	23.49 (2.85)	22.27 (10.37)	16.25 (10.02)
	5-10	10.18 (3.98)	1.12 (0.93)	15.13 (10.94)	15.68 (2.48)	34.37 (36.97)
	10-20	11.11 (6.30)	0.53 (0.37)	22.23 (3.49)	15.16 (2.14)	11.28 (3.67)
	> 20					
Pine 16ys	Whole soil	9.64 (5.04)	0.32 (0.18)	30.23 (3.16)	14.12 (3.24)	8.13 (2.24)
	0.25-2	15.66 (4.96)	0.57 (0.22)	28.04 (2.12)	19.37 (3.34)	12.78 (3.29)
	2-5	5.98 (2.59)	0.19 (0.09)	32.65 (2.27)	11.69 (3.77)	6.13 (1.78)
	5-10	3.05 (0.72)	0.10 (0.03)	32.17 (6.59)	7.45 (2.11)	4.18 (1.85)
	10-20	2.80 (0.16)	0.09 (0.00)	30.19 (0.80)	8.47 (2.02)	4.62 (0.86)
	> 20	2.75 (0.95)	0.08 (0.03)	32.87 (1.33)	8.98 (3.92)	4.49 (2.46)
Pine3ys	Whole soil	2.80 (0.08)	0.06 (0.00)	44.74 (0.33)	5.68 (1.98)	2.19 (0.71)
	0.25-2	3.97 (1.61)	0.12 (0.06)	33.06 (3.11)	8.72 (2.06)	4.47 (1.60)
	2-5	2.91 (0.92)	0.09 (0.05)	33.39 (7.82)	6.45 (0.14)	3.22 (1.00)
	5-10	2.40 (0.96)	0.08 (0.04)	33.31 (6.61)	6.05 (1.10)	3.10 (1.33)
	10-20	3.08 (1.43)	0.06 (0.04)	33.43 (1.85)	5.50 (0.01)	2.79 (0.01)
	> 20					

Table 5.5: Basal respiration rate (μg C-CO_2 g^{-1} aggregate h^{-1}), microbial biomass carbon (μg microbial C g aggregate^{-1}), metabolic quotient (qCO_2) and nitrogen mineralization (mg Nmin kg aggregate^{-1}) of different aggregate sizes from 0-5 cm of the soil of seminatural oak and beech forests, and cultivated pine 40ys, 16ys and 3ys forests.

	Aggregate size (mm)	Oak	Beech	Pine 40ys	Pine 16ys	Pine 3ys
BR	0.25-2	5.37 (2.67)	8.25 (5.05)	7.14 (0.54)	4.51 (3.10)	2.17 (0.91)
	2-5	2.67 (0.70)	5.32 (2.39)	3.84 (1.60)	2.26 (0.72)	1.67 (0.54)
	5-10	2.22 (0.35)	4.59 (2.35)	3.38 (1.41)	2.47 (0.80)	2.76 (2.04)
	10-20	1.90 (0.67)	2.88 (1.97)	3.41 (2.05)	2.76 (1.40)	1.84 (0.67)
	>20	1.67 (0.49)	1.69 (0.31)		2.59 (0.78)	
Nmin	0.25-2	50.72 (15.55)	110.61 (37.04)	77.24 (26.60)	59.02 (60.76)	16.46 (29.84)
	2-5	18.84 (2.88)	50.39 (21.89)	85.47 (39.07)	68.31 (52.33)	64.33 (9.49)
	5-10	24.19 (11.66)	40.89 (23.42)	103.65 (33.28)	72.02 (51.27)	53.69 (11.23)
	10-20	20.82 (10.40)	33.79 (26.58)	51.43 (20.03)	128.76 (46.15)	49.07 (21.93)
	>20	26.15 (1.13)	20.60 (3.72)		32.08 (35.50)	
MBC	0.25-2	321.35 (348.64)	619.92 (508.76)	177.43 (215.19)	298.48 (280.87)	309.51 (283.41)
	2-5	361.75 60.54	890.34 (428.96)	633.88 (254.43)	378.33 (71.67)	658.86 (63.68)
	5-10	336.13 (54.44)	390.14 (146.86)	316.07 (313.56)	301.27 (42.53)	423.42 (236.97)
	10-20					
	>20					
qCO_2	0.25-2	34.73 (30.46)	32.42 (36.61)	122.69 (136.67)	27.32 (24.15)	6.66 (3.14)
	2-5	5.82 (2.25)	5.33 (2.55)	5.08 (1.60)	4.78 (2.89)	1.64 (0.79)
	5-10	6.81 (2.11)	11.36 (1.63)	15.13 (6.77)	8.09 (1.69)	7.70 (5.26)
	10-20					
	>20					

5.4. Discussion

5.4.1. Soil aggregates and soil organic matter

Changes in soil aggregate size distribution were linked to tree species and mechanization. Mean weight diameter was significantly higher in semi-natural (beech and oak stands) than in cultivated mature pine. This may be attributed to differences in annual organic matter inputs (Kavvadias et al., 2001), litter quality (Sariyildiz et al., 2005) and the enmeshing effect of roots and associated mycorrhizal hyphae (Miller and Lodge, 1997), which may contribute to stabilization of soil aggregates.

Mechanization reduced the amount of soil in megaaggregate size classes and significantly increased the amount of macroaggregates and microaggregates in the recently established stand. However, in contrast to findings in agricultural soils (Grandy and Robertson, 2006; De Gryze et al., 2004), 16 years after disturbance the soil physical structure represented by the mean weight diameter was similar to that in mature stands.

Differences in total carbon and nitrogen due to changes in tree species and mechanical site preparation were not detected in the whole soil, because of the relatively high background levels of soil C (Bossuyt et al., 2002) and because site had a greater influence on soil C stocks than tree species (Ladegaard-Pedersen et al., 2005). As regards SOM loss following mechanical site preparation, the data obtained from prior studies in temperate ecosystems was inconclusive. For example, Oliver et al. (2004), found a significant reduction in the concentrations of soil C in the first 10 cm of *Pinus radiata* plantations in which a bulldozer and excavator were used after harvesting, however, Piatek et al. (2003) did not find any significant effects on soil organic C and N in scarified plots of Douglas fir in which mineral soil was exposed by a bulldozer with a blade.

Despite the lack of change in total soil C and N, the POM fraction was significantly affected by mechanical site preparation in *Pinus radiata* cultivated forests. Mechanization was also found by Mubarak et al. (2005) to have such an effect. Reduction of the POM-C fraction induced by mechanical site preparation may also affect the aggregate size distribution, because this physically uncomplexed OM is highly susceptible to breakdown when the soil is disturbed at a very rapid turn-over rate (Henderson et al., 2004; Baisden et al., 2002).

The functional role of POM-C fraction in soil aggregation was reflected in this study by the relationship between MWD and POM-C:SOM. Pikul et al. (2007) also found a relationship between aggregation and fine POM-C:SOM. The latter authors concluded, in a similar way as Gale et al. (2000b), that in undisturbed soils, POM is derived primarily from roots, and that new microaggregates are formed around decomposing pieces of POM inside macroaggregates.

5.4.2. Soil aggregate C and dynamics

The concentrations of SOC did not increase with increasing aggregate size class in any of the studied forests, except in the pine3ys stand. It appears that the concept of aggregate hierarchy proposed for agricultural soils according to which microaggregates are bound together into macroaggregates by transient binding agents (Tisdall and Oades, 1982), only occurred in the recently mechanized stand. Mechanization in this stand may produce similar effects to those undergone in agricultural soils. Harvesting and mechanical site preparation induce more intense

soil disturbance than agricultural practices, but each of these occurs only once in the rotation period while the disturbance induced by agricultural practices is repeated every year.

The aggregation in mature forest soils may be linked to the POM fraction, as reflected by higher concentrations of POM-C in macroaggregates and lower C:N ratios in this POM fraction. Decomposition of POM is thought to release microbial exudates that stabilize macroaggregates (Oades, 1984, Six et al., 2000a) and, in this way, new microaggregates are formed inside the existing macroaggregates (Gale et al., 2000b). The significant relationships found in the present study between (i) POM-C and respired CO_2 and (ii) POM-N and mineralized N suggest that biological activity was more intense in this POM fraction than in any other one. Magid and Kjaegaard (2001) reached the same conclusion, with results similar to present ones. Moreover, it must be remembered that the POM fraction is the fraction most affected by mechanization.

In mature forests, macroaggregates (0.25-2 mm) showed higher rates of soil respiration, metabolic quotients and nitrogen mineralization (not in pine 40ys) than megaaggregates. In less disturbed ecosystems, the organic matter within macroaggregates may be the free light POM fraction (Cambardella and Elliot, 1993) and these macroaggregates may be of special interest in aggregate dynamics. However, in more disturbed ecosystems (pine 16ys and pine 3ys), the significant loss of this fraction may alter the aggregate dynamics, because the same trends were not detected in these stands.

5.5. Conclusions

In temperate forest ecosystems, there are certain problems related to the cultivation of rapidly growing exotic species and intensive forest management with mechanized methods. Importantly, the present results show that indicators for sustainable forest management related to soil organic matter should not only be assessed in terms of total C stocks but also with respect to organic matter composition and stability.

The consistent relationship between POM-C:SOM of the whole soil and MWD led us to conclude that aggregate formation may be directly related to POM dynamics in these forest soils, independently of tree species. Because POM has been shown to be a labile fraction of SOM (relationship with soil respiration and nitrogen mineralization), an effort should be made to conserve this OM pool in sustainable forest management in order to maintain long-term ecosystem functioning.

The intensive mechanization during establishment of *Pinus radiata* plantations resulted in physical disruption of soil structure and a significant reduction in the POM fraction in the top 5

cm of soil. Although the MWD and the amounts of POM had recovered 16 years after disturbance, the quality of POM fraction was still significantly different.

Finally, it appears that macroaggregates (0.25-2 mm) may play an important role in aggregate dynamics in mature forests, independently of tree species.

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5.6. References

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6. Landscape-level patterns of soil microbial community level physiological profiles in temperate forest ecosystems

Soil microbial communities govern important biogeochemical processes, including organic matter dynamics, and differences in microbial communities associated with plant species are thought to result from variations in the quantity and quality of carbon inputs. However, landscape-level differences should be considered in forest ecosystems because plant litter can vary widely in its chemical constituents at this scale. Forest ecosystems that differ in tree species (Quercus robur L., Fagus sylvatica L., Quercus ilex L. and Pinus radiata D.Don) were studied in order to evaluate the amount and quality of forest floor and its influence on the function of the soil microbial community over a wide geographical range (the Ibaizabal basin) in the Basque Country.

Samples of forest floor and soil (0-30 cm and 30-60 cm) were collected from 42 temperate forests. Forest floor properties were determined, by chemical and proximate analysis, and community-level physiological profiles (CLPP) were constructed by use of a MicroResp™ assay. Canonical variate analysis (CVA) of the forest floor data differentiated holm oak and oak forest floors from beech and pine forest floors, but forest floors from beech and pine could not be distinguished. However, CVA of the CLPP data differentiated microbial communities from both soil depths (0-30 cm compared with 30-60cm) and tree species. CLPP were highly correlated with soil properties related to organic matter, such as carbon, nitrogen, phosphorus and water holding capacity, and soil properties related to parent material, such as calcium concentration.

6.1. Introduction

Soil microbial communities mediate many biogeochemical processes that are central to ecosystem functioning. Balser and F

irestone (2005) found that although microbial biomass was not affected by soil transplanted from grassland to a conifer forest ecosystem, characteristics of microbial communities, such as substrate utilization patterns, were related to soil processes, such as *in situ* N mineralization. Griffiths et al. (2000) also found that as soil microbial diversity decreased with intensive agricultural management practices, nitrification also decreased and decomposition increased. Therefore, in order to study the soil ecosystem or to observe the impact of human activities on the ecosystem, it is essential to characterize soil microbial communities.

The Biolog® system of community-level physiological profiling (CLPP) of the microbial community has been widely used to characterize microbial communities on the basis of a pattern of substrate utilization in 96-well microtitre plates (Garland and Mills, 1991). The Biolog® method has been used to characterize microbial communities associated with tilled agricultural soils (Lupwayi et al., 2001), plant rhizospheres (Grayston et al., 1998) and tree species (Priha et al., 2001). In fact, Priha et al. (2001) were not able to detect any difference in the function of microbial communities in forest soils developed under Scots pine, Norway spruce or Silver birch with the Biolog® system. However, differences in the composition of soil microbial communities were reflected by PLFA analysis (Priha et al, 2001). Problems related to culturability and growth of bacteria have generated some criticism of the Biolog® method (Konopka et al., 1998; Campbell et al., 2003; Ritz 2007). Bearing this in mind, the MicroResp™ assay may be a novel approach that could provide meaningful and comparable data for substrate induced responses of soil microorganisms. The MicroResp™ method combines the advantages of Biolog® method, in which microtitre-based detection plates are used, with the ability to measure release of carbon dioxide from the whole microbial community present in the soil and not just from the culturable bacteria. Community-level physiological profiles (CLPP) obtained from MicroResp™ are used in this study as a measure of functional diversity of the whole microbial community.

The majority of soil microbial community characterizations have been conducted over small spatial scales or at relatively few sites. However, landscape-level differences may be considered in forest ecosystems as many important parameters that affect microbial community differ greatly at this scale; e.g. (i) plant litter can vary widely in its chemical constituents, (ii) plant community composition can also vary at this scale and may directly influence microbial community composition (Myers et al., 2001). In New Zealand, Stevenson (2004) found evidence that pine

forests and native forests differed in substrate utilization patterns constructed with catabolic response profiles (CRP) on the basis of the method of Degens and Vojvodic-Vukovic (1999). Notwithstanding these observations, understanding of the manner in which the broader soil microbial community varies with plant community composition at a landscape-level in temperate ecosystems is limited.

The aims of the present study were: i) to elucidate whether differences in litter quality under *Quercus robur*, *Fagus sylvatica*, *Quercurs ilex* and *Pinus radiata* forests were consistent at a landscape level, ii) to evaluate whether community level physiological profiles (CLPP) established with whole microbial community of the soil could be used at this scale to differentiate different forests and iii) to examine the relationship between microbial CLPP and selected forest floor and soil properties.

6.2. Materials and Methods

6.2.1. Sites and sampling

Forty-two forest stands were sampled during the summer of 2006 within a geographical range of approximately 450 km^2 in the Ibaizabal basin, in the Atlantic zone of the Basque Country (43º 07´N 2º 51´W). The territory is steeply sloping, with altitudes of between <50 m near the coast and 1200 m at the mountain tops, at distances of <30–50 km, resulting in frequent slopes of 40–60%. Rainfall ranges from 800–1200 mm and the heaviest rainfalls occur between September and May. Limestone, loam and dolomites predominate in the territory, as do Leptosols, Regosols and Cambisols (FAO, 1988). The upper horizons generally show advanced development, sometimes surpassing 50 cm.

All sampled forests conformed with those used in the National Forest Inventory (IFN, 2005) and represented semi-natural and mature cultivated forests in the Atlantic region of the Basque Country. *Pinus radiata* D.Don forests (12 plots) were productive cultivated monospecific stands. All of them were characterized by being older than 20 years, and taking into account the forest management history, these forests were probably not managed with heavy machinery, so soil disturbance may be minimal at stand establishment. *Quercus robur* L. (14 plots), *Fagus sylvatica* L. (9 plots) and *Quercus ilex* L. forests (7 plots) represented semi-natural temperate forests. *Quercus robur* forests (namely oak) represented mixed forests dominated by oak, chestnut and birch, with *Erica vagans* L., *Ulex europaeus* L., *Rubus ulmifolius* Schott and *Ilex aquifolium* L. in the understorey. Until the mid 1940s, *Fagus sylvatica* (beech) forests were characterized by pollarded beeches used for firewood and charcoal and not managed in the last decades. *Quercus*

ilex forests (namely holm oak) were semi-natural forests established in very shallow soils, also managed for charcoal production some decades ago and not managed in the past few decades.

Samples were collected from 5 by 5 m plots following a systematic sampling in a criss-cross pattern. Ten cores (40 mm diam. by 1 m depth) were sampled on two lines that crossed the centre of the NFI plot. Soil samples were divided into 0-30 cm and 30-60 cm subsamples, composited for each depth and split into field-moist and dry portions. The field-moist portion was sieved to < 2 mm to remove large woody debris and stones, and maintained at 4ºC. The dry portion was dried at 30ºC and also sieved to <2 mm. Additionally, 3 forest floor samples were collected at 3 sampling points, where forest floor quantity was representative of the plot. Forest floor was sampled with a 30 x 30 cm template, air-dried, weighed and ground to pass a 1 mm mesh for proximate analysis and < 0.5 mm for chemical analysis.

6.2.2. Forest floor analyses

Organic C and total N were analysed in a LECO CNS 2000 analyser (LECO Corporation, MI, USA). Total concentrations of P, Ca, Mg, K and Na were determined in aqueous extracts after wet digestion with nitric-perchloric acid by Inductively Coupled Plasma Emission Spectroscopy (Varian Iberica S.L., Barcelona, Spain). The water-soluble carbohydrates (WSC) were determined colorimetrically by the anthrone method in water extracts, according to Deriaz (1961). Acid-detergent fibre (ADF) and acid-detergent lignin (ADL) were determined with an ANKOM^{220} fiber analyzer (ANKOM Technology, NY, USA) (van Soest et al., 1991). The cellulose fraction was calculated as (CEL=ADF−ADL) and the ADL fraction was referred to as lignin, although it included recalcitrant by-products of decomposition. Ash (ASH) was determined after combustion for 4 h at 550 °C.

6.2.3. Soil chemical and physical analyses

Total organic C and N were measured with a LECO CNS 2000 autoanalyser (LECO Corporation, MI, USA). Soil pH was determined in water (1:2.5) and cations (K, Ca, Mg, Na) after extraction with NH4-acetate 1.0 M. Phosphorus was extracted with sodium bicarbonate (0.5 M; pH 8.5) and determined by colorimetry in an UV-Visible spectrophotometer Varian Cary 100 (Varian, Inc., Palo Alto, Ca.) (Watanabe and Olsen 1965; AOAC, 1980). Cation exchange capacity (CEC) was determined after saturation of the sample with 1N sodium-acetate. The Na was removed with a solution of NH4-acetate (1N) and Na was determined on a Varian Spectra 220 FS (Varian, Inc., Palo Alto, Ca.) by flame atomic absorption photometry (AOAC, 1980). Extractable cations were also determined by atomic absorption photometry with the above mentioned apparatus.

Aluminium was extracted with a $BaCl_2$ solution (0.6N) and determined by titration with sodium hydroxide and phenophtalein (Mosquera, 1990).

Soil particle analysis was performed by laser diffractometry, with a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK), and soil water holding capacity (WHC) was determined by water content at -33KPa in standard tension plates (Klute, 1986).

6.2.4. MicroResp™ assay

The MicroResp™ methodology used in the study is described in Campbell et al. (2003). In brief, the system consisted of two microtitre wells placed face to face and connected with MicroResp™ seal. Freshly collected soils were preadjusted to 40 % of the water holding capacity so that after the carbon source solution was added, the moisture content was 60 % of the soil water holding capacity. The carbon sources used in the present study were selected from those described by Stevenson et al., 2004, on the basis of their ecological relevance to the soil microbial community (e.g. plant root exudates) and on their ability to be dissolved in water. The 15 carbon substrates included in the assay were: two amines (L-glutamine and N-acetyl-D-glucosamine), four amino acids (L-arginine, L-lysine, L-cysteine and L-phenylalanine), two carbohydrates (D-glucose and D-fructose), six carboxylic acids (L-ascorbic acid, citric acid, α-ketoglutaric acid, succinic acid, pantothenic acid and coumaric acid) and one phenolic acid (vainilline). The substrates were used at the following concentrations: amino acid and amine solutions, 15 mM, carboxylic acid solutions, 100 mM and carbohydrate solutions, 75 mM.

Soils were preincubated in the dark for 5 days at 28ºC and then added to deep wells containing carbon sources. Each well was then immediately sealed with the detection plate. This plate consisted of a gel containing a pH indicator dye (Rowell, 1995), which causes a colour change with absorption of CO_2. For colorimetric detection of CO_2 produced by soil microorganisms, the detection plate was read immediately before and 6 h after incubation at 28ºC, in a microtitre plate reader (Zenyth 3100, Anthos Labtec Instruments, Salzburg, Austria), at 570 nm. The absorbances recorded before and after incubation period were normalized and converted to the headspace CO_2 concentrations by use of the corresponding calibration curve. A calibration curve for absorbance plotted against headspace equilibrium CO_2 concentration was determined by equilibrating dye solutions at different CO_2 concentrations prepared with standard gas mixtures (Fig 1). The best-fit curve was determined by regression analysis and the result was as follows (R^2= 0.99, $P < 0.001$): $\%CO_2 = A + B/x$, where A= -6.66, B= 7.34 and x was the absorbance data after 6 hours of incubation.

The Shannon diversity index was used as a practical indicator of the overall measure of microbial functional diversity (Degens et al., 2001).

6.2.5. Statistical analyses

One-way analyses of variances were performed to determine differences in forest floor and soil chemical parameters, and protected least-significant differences were calculated at the 5% level as a Post Hoc test to show differences in the characteristics among the species. The community level physiological profiles (CLPP) were first analysed by one-way analysis of variance ($P< 0.05$) to determine whether individual carbon substrate responses differed between forest types, and a Student t-test was used to determine whether individual carbon substrate responses differed at different soil depths ($P< 0.05$). Forest floor properties and CLPP data were then used in separate canonical variate analyses to determine whether forest samples differed in their overall properties at a landscape level. The ordination coordinate loadings were observed to identify which properties accounted for most of the discrimination and were compared by a protected least-significant difference mean separation test. Correlation matrix was used to determine statistical associations between forest floor and soil chemical and physical properties and CLPP responses. The associations were considered significant at P <= 0.05 after application of the Bonferroni method for the adjustment of probabilities (González-Arias et al., 2006). All statistical analyses were carried out with SPSS 13.0 for Macintosh (SPSS, 2004).

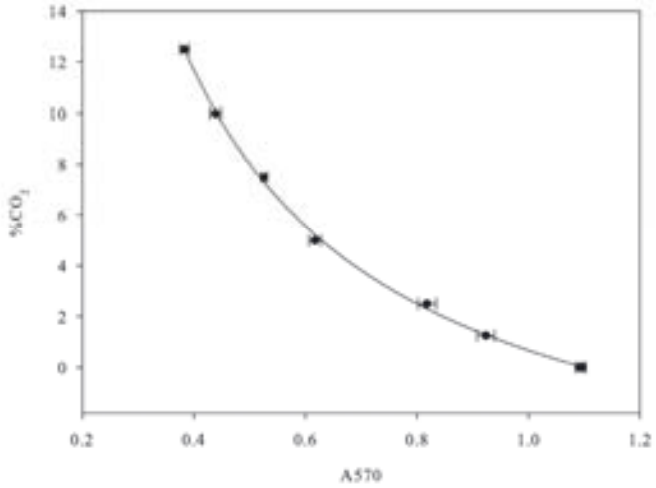

Figure 6.1: Calibration curve for absorbance (A570) versus percentage of CO_2 for standard gas mixtures.

6.3. Results

6.3.1. Soil characteristics

Forests varied significantly in soil chemical characteristics (Table 6.1). At 0-30 cm depth, soils under all types forest were acidic, however the highest pH values were under holm oak forests and the lowest under pine forests. Consequently, holm oak had the highest concentration of Ca in soil ($P < 0.05$) and pine forests showed the lowest ($P < 0.05$). Holm oak forests also showed significantly higher concentrations of carbon, nitrogen, phosphorus and potassium than beech, oak and pine, as well as higher cation exchange capacity; and, at the same time, beech showed higher concentrations of carbon, nitrogen and phosphorus than oak and pine forests.

However, the forests under study did not differ significantly in terms of soil physical characteristics. The only significant difference in the relative portions of particles in the forests was found in the clay content, and was lower in beech forests than in the others. Water holding capacity (WHC) tended to be higher in beech and holm oak forests than in oak and pine forests.
Soil depth had a significant effect on almost all soil properties. Holm oak forests were developed on very shallow soils, making it impossible to obtain samples from the 30-60 cm soil layer. The decrease in soil organic matter with depth occurred as follows; 70 % in oak forests, 50 % in pine forests and 40 % in beech forests. In contrast, beech forests showed the greatest reduction in nitrogen concentration with depth, almost 60 % less nitrogen was found in 30-60 cm depth layer in beech forests, while oak and pine forests showed reductions in nitrogen of around 40 %, with depth.

Table 6.1: Analysis of variance results for the effects of tree species on physical and chemical properties of soil layers (0-30 cm and 30-60 cm depth). Values are means ± standard error.

		Oak (n=14)	*Beech (n=9)*	*Pine (n=12)*	*Holm Oak (n=7)*	*F-value*
0-30 cm	Coarse Sand, %	4.09 ± 1.04	4.34 ± 1.06	3.27 ± 0.73	5.05 ± 1.30	*ns*
	Fine Sand, %	14.83 ± 2.35	21.92 ± 3.47	15.26 ± 3.15	18.46 ± 3.90	*ns*
	Silt, %	44.62 ± 1.99	50.78 ± 3.53	46.29 ± 1.96	42.26 ± 2.47	*ns*
	Clay, %	36.46 ± 2.80	22.92 ± 3.28	35.18 ± 2.88	34.24 ± 6.87	3.08*
	WHC	31.52 ± 2.70	46.92 ± 9.19	35.33 ± 2.56	42.50 ± 5.20	2.18
	pH	5.42 ± 0.28	4.58 ± 0.43	4.48 ± 0.15	6.15 ± 0.29	4.98**
	C, %	1.66 ± 0.43	2.72 ± 0.35	2.11 ± 0.49	6.52 ± 1.27	9.92***
	N, %	0.19 ± 0.04	0.38 ± 0.10	0.18 ± 0.02	0.57 ± 0.10	7.48**
	C/N	8.60 ± 0.75	10.34 ± 0.81	10.53 ± 1.29	11.43 ± 0.84	*ns*
	P, ppm	1.23 ± 0.40	1.70 ± 1.10	0.62 ± 0.18	3.90 ± 1.56	3.29*
	C/P	6.13 ± 3.20	3.49 ± 1.53	4.91 ± 1.92	2.57 ± 0.76	*ns*
	Ca, meq/100	6.30 ± 2.05	11.66 ± 6.27	2.74 ± 0.71	23.49 ± 6.03	4.89**
	Mg, meq/100	0.49 ± 0.10	0.63 ± 0.22	0.44 ± 0.12	0.98 ± 0.17	*ns*
	K, ppm	76.15 ± 8.79	85.50 ± 9.81	94.20 ± 11.38	151.00 ± 25.75	4.89**
	CEC, meq/100	16.71 ± 1.94	20.11 ± 2.56	17.83 ± 1.91	31.65 ± 4.93	4.96**
	Al, meq/100	3.80 ± 0.96	7.06 ± 2.09	6.11 ± 1.25		4.93**
	Sat. Al, %	49.89 ± 10.72	72.00 ± 8.11	60.30 ± 8.43		7.95**
30-60 cm	Coarse Sand, %	3.26 ± 0.64	1.98 ± 0.57	2.70 ± 0.59		*ns*
	Fine Sand, %	13.46 ± 2.64	15.22 ± 2.25	15.80 ± 2.93		*ns*
	Silt, %	45.00 ± 2.49	49.53 ± 4.22	46.12 ± 1.42		*ns*
	Clay, %	38.26 ± 3.46	33.26 ± 3.10	35.39 ± 3.27		*ns*
	WHC	27.44 ± 1.80	38.18 ± 4.53	32.58 ± 1.61		4.67*
	pH	5.38 ± 0.34	4.86 ± 0.49	4.73 ± 0.12		*ns*
	C, %	0.52 ± 0.05	1.65 ± 0.27	1.03 ± 0.11		19.49***
	N, %	0.11 ± 0.01	0.17 ± 0.02	0.12 ± 0.01		4.62*
	C/N	5.06 ± 0.57	9.42 ± 0.36	8.53 ± 0.90		9.19**
	P, ppm	0.47 ± 0.17	0.08 ± 0.05	0.74 ± 0.58		*ns*
	C/P	2.94 ± 1.52	10.42 ± 0.07	13.67 ± 5.06		*ns*
	Ca, meq/100	4.09 ± 1.14	5.41 ± 2.57	2.84 ± 0.88		*ns*
	Mg, meq/100	0.39 ± 0.12	0.27 ± 0.08	0.59 ± 0.21		*ns*
	K, ppm	56.42 ± 6.31	58.60 ± 8.91	74.82 ± 7.04		*ns*
	CEC, meq/100	13.43 ± 1.34	17.25 ± 2.38	15.72 ± 1.16		*ns*
	Al, meq/100	4.10 ± 0.75	5.97 ± 2.26	4.86 ± 0.76		*ns*
	Sat. Al, %	62.25 ± 8.33	72.00 ± 21.52	59.18 ± 8.56		*ns*

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

6.3.2. Forest floor characteristics

The lowest forest floor mass was found in oak forests and the highest accumulation of forest floor in beech forests (Table 6.2). Although analysis of variance of individual forest floor properties revealed few significant differences beneath different types of forest, oak forests were characterized by significantly higher concentrations of Mg than beech, holm oak and pine forests; and beech forests by the highest mean values of CEL and lignin/N. Holm oak showed higher concentrations of Ca and C/P ratios, and pine forests showed intermediate values of all forest floor properties.

Table 6.2: Analysis of variance results for the effects of tree species on forest floor properties. Values are means ± standard error.

	Oak (n=14)	Beech (n=9)	Pine (n=12)	Holm Oak (n=7)	F-value
mass, g m^{-2}	466.11 ± 83.16	1226.51 ± 264.90	806.50 ± 137.28	893.53 ± 246.80	3.70*
C, %	49.02 ± 1.39	49.44 ± 1.81	49.34 ± 1.94	51.26 ± 1.17	ns
N, %	1.29 ± 0.03	1.44 ± 0.10	1.23 ± 0.08	1.36 ± 0.06	ns
P, mg g^{-1}	0.65 ± 0.10	0.69 ± 0.07	0.66 ± 0.07	0.56 ± 0.02	ns
K, mg g^{-1}	4.14 ± 0.33	2.96 ± 0.56	3.56 ± 0.61	2.17 ± 0.16	3.16*
S, mg g^{-1}	1.29 ± 0.07	1.46 ± 0.11	1.30 ± 0.07	1.31 ± 0.06	ns
Ca, mg g^{-1}	13.01 ± 1.52	15.12 ± 2.49	9.17 ± 2.59	18.59 ± 2.51	2.73
Mg, mg g^{-1}	1.66 ± 0.15	1.09 ± 0.05	1.20 ± 0.14	0.99 ± 0.11	5.61**
ASH, %	6.65 ± 1.02	9.69 ± 2.51	7.55 ± 1.33	6.63 ± 1.56	ns
ADF, %	57.08 ± 3.11	60.86 ± 3.78	54.91 ± 4.04	53.79 ± 3.16	ns
CEL, %	23.18 ± 1.03	26.32 ± 2.62	22.75 ± 1.46	21.03 ± 0.79	ns
Lignin, %	33.90 ± 2.66	34.56 ± 2.78	32.16 ± 3.34	32.77 ± 2.81	ns
WSC, mg g^{-1}	6.19 ± 0.65	5.61 ± 0.61	5.59 ± 0.69	6.97 ± 1.56	ns
C/N	38.79 ± 1.72	35.31 ± 2.19	40.81 ± 1.96	38.40 ± 1.91	ns
C/P	919.01 ± 108.63	753.24 ± 68.94	823.45 ± 82.93	945.34 ± 29.73	ns
lignin/N	18.35 ± 1.03	19.07 ± 2.35	19.26 ± 1.89	15.80 ± 1.00	ns
lignin/cel	0.41 ± 0.02	0.43 ± 0.03	0.41 ± 0.03	0.41 ± 0.03	ns

* $P < 0.05$ and ** $P < 0.01$

Nevertheless, canonical variate analysis on forest floor properties successfully discriminated forest floors from different tree species, with 84 % of variance explained with the first two canonical variate functions (Fig 6.2). Forest floors from oak and holm oak forests were discriminated on CV1 (F= 6.20, $P < 0.001$) from beech and pine forests. On CV2, forest floors from oak and holm oak forests were discriminated from each other and from the other forests (F= 5.54, $P < 0.05$). Forest floors from beech could not be discriminated from pine forests with these properties. Analysis of individual loadings of forest floor properties on CV1 indicated that Mg, P, K, CEL and lignin/N ratios mainly accounted for the differences, and C/P and C/N ratios mainly accounted for the discrimination on CV2 of oak forests from holm oak forest floors.

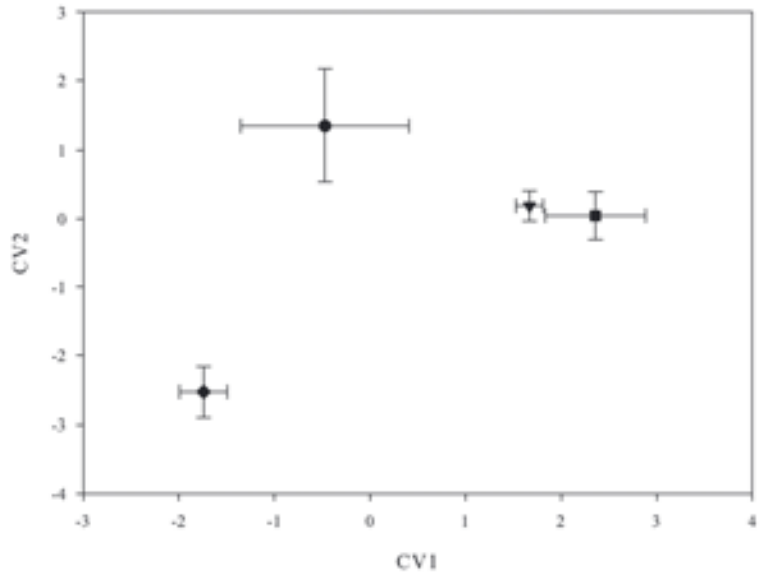

Figure 6.2: Discrimination of forest floor properties. Closed symbols represent 0-30 cm layer samples and open symbols 30-60 cm samples. (●) oak, (▼) beech, (◆) holm oak and (■) pine forests. Values represent means and error bars represent standard error of the means.

6.3.3. Community level physiological profiles (CLPP)

The response of soil microbial community to all individual carbon substrates decreased substantially with soil depth (Fig 6.3.). In both soil layers and in all forest types, the soil microbial community responded most to carboxylic acids, mainly to citric acid, α-ketoglutaric acid and ascorbic acid. On the other hand, L-arginine and L-cisteine, as individual carbon substrate, produced a negative response in soil microbial community in both layers as they evolved less CO_2 than in basal respiration.

In the 0-30 cm soil layer, beech and holm oak forests showed significantly greater responses to all carbon substrate groups than oak (Fig 6.3a). Pine forests showed intermediate responses between oak and beech and holm oak forests. The composite response of the 4 amino acids studied did not reflect any microbial activity in any forest type except in holm oak. In the 30-60 cm soil layer, beech forests showed the highest response to all carbon substrate groups. Oak and pine forests differed in the response to carbohydrates and amines but had similar responses to amino acids, carboxylic acids and phenolic acids (Fig 6.3b).

The Shannon diversity index, which is a practical indicator of overall measure of microbial diversity (Degens et al., 2001), did not show any differences among forest types in the 0-30 cm soil layer nor in the 30-60 cm soil layer. The values of the Shannon diversity index were similar in

both soil layers in beech and pine forests, but in oak forests it decreased significantly in functional diversity with depth ($t = 3.25$, $P < 0.001$) (data not shown).

Multivariate discriminant analysis of the entire CLPP data set indicated significant separation of the groupings both by soil depth (0-30 cm compared with 30-60 cm) and by forest type (oak, beech, holm oak or pine) (Fig 6.4). The first two canonical variate functions (CV) accounted for 76 % of the total variance. Microbial carbon utilization patterns of different forest types were discriminated on CV1 ($F = 10.32$, $P < 0.0001$). Holm oak forests differed substantially from other forest types in terms of their utilization patterns, with consistently higher coordinates on CV1. Oak forests were also different from beech, pine and holm oak forests on CV1, with the lowest coordinate values on this axis. Pine and beech forests showed similar scores on the CV1 axis, however both groups may be separated on CV2.

Soil microbial communities from 0-30 cm and 30-60 cm depth layers were discriminated on CV2 ($t = -5.01$, $P < 0.001$) with communities from 30-60 cm showing higher coordinate values on this axis. Analysis of individual loadings on individual carbon sources on CV1 indicated that differences in microbial utilization of carboxylic acids and N-acetyl-D-glucosamine mainly accounted for the discrimination on this axis and microbial utilization of D-fructose and L-glutamine mainly accounted for the discrimination on CV2.

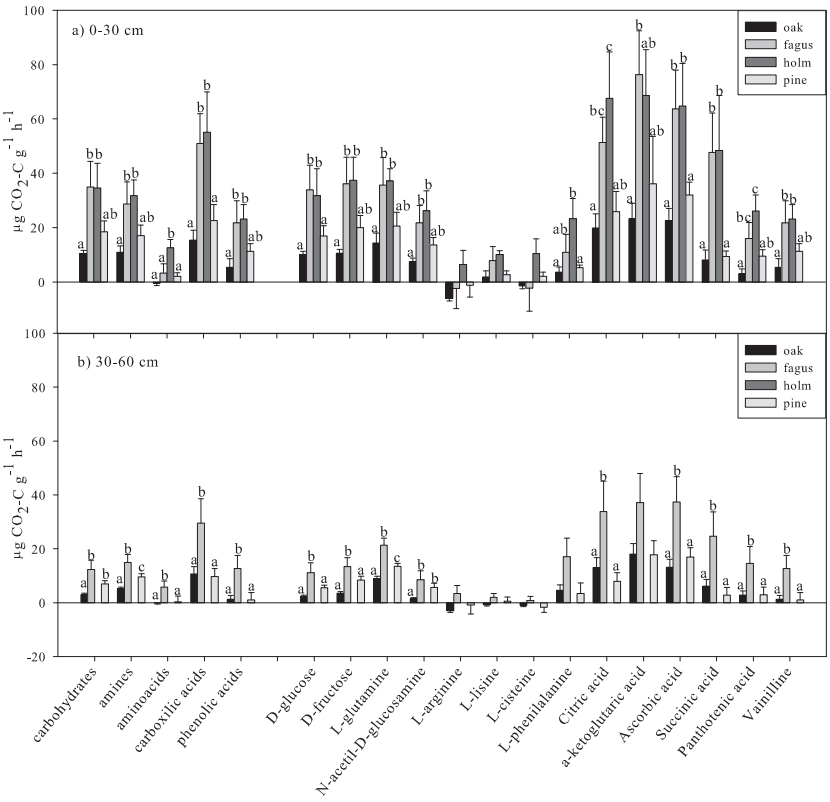

Figure 6.3: Responses of different forest soils a) 0-30 cm and b) 30-60 cm to individual carbon substrates and carbon groupings. Error bars represent standard error of the mean. Substrates with lower case letters above indicate a significant difference between forest types and bars followed by different lower case letters indicate significant difference at $P < 0.05$

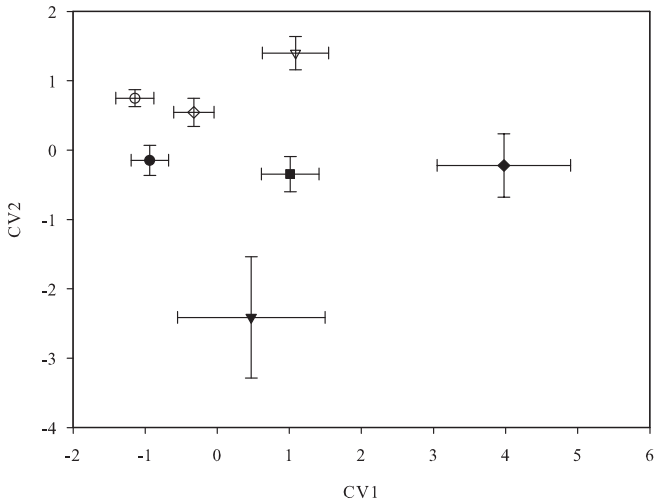

Figure 6.4: Discrimination of microbial communities from the forest soils based on CLPP profiles. Closed symbols represent 0-30 cm layer samples and open symbols 30-60 cm samples. (●) oak, (▼) beech, (◆) holm oak and (■) pine forests. Values represent means and error bars represent standard error of the means.

6.3.4. Relationships between forest floor, soil and microbial utilization patterns

Pearson correlation matrix with probabilities adjusted with Bonferroni method was performed to gain an insight into the relationships between forest floor properties, soil physical and chemical properties and soil microbial utilization patterns. The only significant association between forest floor and soil properties was related to nitrogen. The concentration of nitrogen in the forest floor was positively related to soil organic matter, and concentrations of nitrogen, phosphorus and calcium of the mineral soil (Table 6.3). However, both forest floor and soil properties exerted a positive influence on the response of the soil microbial community. Forest floor mass exerted a positive influence on the microbial response to carbohydrates while calcium concentration was positively related to the activity of carbohydrates, amines and carboxylic acids, and nitrogen concentration influenced the response of the microbial community to phenolic acids.

However, microbial utilization of carbon substrate groups was more closely related to soil properties than to forest floor properties. The microbial response of carbohydrates, amines and carboxylic acids were positively related to soil organic matter properties such as concentrations of carbon, nitrogen and phosphorus. Moreover, the microbial responses to these carbon substrate groups were correlated with water holding capacity (WHC), although this property was also related to soil organic matter content. The microbial response to phenolic acids was related to calcium concentration and cation exchange capacity as well as to soil organic matter. The microbial response to amino acids or Shannon diversity Index did not show any significant association with any forest floor or soil property (Table 6.3).

6.4. Discussion

Ecosystems that differ in terms of tree species may produce litter with chemically distinct substrates that may drive pulse microbial growth and therefore, organic matter dynamics differently. The forest ecosystems under study did not differ substantially in litter chemistry at this landscape-level, implying that the broad differences in litter chemistry noted by other scientists among tree species (Lorenz et al., 2000; Myers et al., 2001) (i) may be reduced when the spatial scale is broaden as differences in soil chemistry and availability of nutrients for all the species will spread the variance of this parameter, or that (ii) differences in litter chemistry may be

Table 6.3: Correlation matrix between CLPP responses (C substrate groups), forest floor properties and soil physico-chemical properties in 0-30 cm soil layer.

		Microbial community					Forest floor properties													Soil physico-chemical properties							
		SI	Carboh	Amines	AA	Carbox	Phen	Mass	CEL	LIG	WSC	P	K	Ca	Mg	N	MO	C/N	C/P	LIG/N	Clay	WHC	pH	MO	N	C/N	P	Ca	Mg
Microbial community	SI																												
	Carboh																												
	Amines		.942																										
	AA		.582	.637																									
	Carbox		.915	.891	.612																								
	Phen		.737	.811	.728	.816																							
Forest floor properties	Mass		.557																										
	CEL																												
	LIG																												
	WSC																												
	P																												
	K																												
	Ca		.638	.602		.692																							
	Mg																												
	N					.550	.593																						
	MO												-.55																
	CN															-.78													
	CP											-.83																	
	LIG/N								.770							-.61		.659											
Soil physico-chemical properties	Clay																												
	WHC		.604	.614		.672																							
	pH																												
	MO		.702	.689		.703	.684									.670						.654							
	N		.747	.729		.752	.690									.687						.833		.987					
	C/N																												
	P		.691	.625		.638										.587								.949	.949				
	Ca					.622										.608						.781	.765	.764	.885		.588		
	Mg																					.667	.688	.582	.762			.854	
	K																							.597					
	CEC					.580	.660															.696		.860	.845			.794	.704
	Al																						-.87	.709		.751		-.69	

Microbial community; SI = Shannon diversity Index, Carboh = carbohydrates (µg C-CO_2 g^{-1} h^{-1}), Amines = amines (µg C-CO_2 g^{-1} h^{-1}), AA = amino acids (µg C-CO_2 g^{-1} h^{-1}), Carbox = carboxilic acid (µg C-CO_2 g^{-1} h^{-1}), Phen = phenolic acids (µg C-CO_2 g^{-1} h^{-1}), Forest floor properties; Mass = Forest floor mass (g m^2), CEL = cellulose (%), LIG = lignin (%), WSC = water soluble carbohydrate (mg g^{-1}), P, K, Ca, Mg = phosphorous, potassium, calcium, magnesium (mg g^{-1}), N, MO = nitrogen and organic matter (%), Soil properties; Clay = clay (%), WHC = water holding capacity (%), MO = organic matter and nitrogen (%), P = phosphorous (ppm), Ca, Mg = calcium and magnesium (meq/100), K = potassium (ppm), CEC = cation exchange capacity (meq/100), Al = aluminium (meq/100).

more consistent in initial decomposition stages of fresh litter and converged to low substrate quality in late stages of decomposition with subtle differences among tree species (Entry and Emmingham, 1998).

In general, soil microbial activity is governed by the availability of C and N, both of them are assumed to be related to litter quality, water and O_2 (West and Sparling, 1986). In this study, water and O_2 limitation were overcome by using soils at 60 % of WHC and enough space to ensure that conditions in the wells did not become anaerobic. Therefore, the availability of C and N should have determined the soil microbial activity in this study. It is therefore not surprising that the 0-30 cm depth layer exhibited higher functional activity than 30-60 cm layer, as the latter may have less energetically favourable substrates for microbial growth and less N with a narrow C/N that indicates relatively stable OM. Differences in soil microbial community composition among forest soil horizons have been noted in other studies involving PLFA analysis (Bååth, 1981; Zvyagintser et al., 1993).

CLPP data showed that microbial communities were more likely to metabolize carboxylic acids, in all forest types and in both soil layers. This same feature was also observed by Stevenson et al. (2004) when comparing pasture and forest soils in New Zealand. This is probably because forest exudates contain a high content of carboxylic acids with respect to crops (Nardi et al., 2002). Furthermore, these organic acids play an important role in soil genesis and plant physiology, because they affect 1) the weathering of primary minerals, 2) the transport of metals through the profile, 3) the availability of plant nutrients, 4) complexation of elements with phytotoxic effects and 5) the exchange of regulatory signals between soil and plants (Certini et al., 2000).

In addition to the quality of C source, microbial community function may also be affected by the quantity of the substrate (Griffiths et al., 1999). The concentration of the amino acids used (15 mM) in this study appeared too high to give heterotrophic response in all forest types except in holm oak forests. The fact that holm oak forests showed significantly higher soil concentrations of nitrogen than other forest types may influence the response of the microbial community. Variations in substrate utilization patterns in the upper forest soil layer may be associated with specific tree species as revealed by CVA analysis. Holm oak were more likely to metabolize ascorbic and citric acid and less likely to metabolize ∝-ketoglutaric and succinic acid. The opposite was true for oak forests. One factor controlling these differences in microbial communities may be the differences between tree species in terms of rhizosphere C (Lamarche et al., 2007). However, these results may also be explained by the interaction between differences in microbial community composition and soil conditions, making it difficult to discern whether changes in microbial utilization patterns are due to tree species.

The properties of soils beneath different types of forest varied consistently, probably in relation to the geology of the parent material, which determines site nutrient capital and particularly, site physical properties (Lamarche et al., 2007). Oak and pine forests shared similar soil properties, with lower concentrations of carbon, nitrogen and phosphorus in the upper soil layer than in beech forests. Holm oak was characterized by the highest pH and concentrations of calcium.

Contrary to the response observed by Grayston et al. (2003) and White et al. (2005), the variations in microbial C utilization patterns were not related to soil pH. Soil acidity has been linked to a decrease in the availability of carbon to microbial communities (Bååth et al., 1995), however pH values of forest soil studied ranged from 4.5-6.1. As the acidic bacterial communities are adapted to this narrow range, some other characteristic may be important in differentiating their activity (Bååth, 1996; Andersson and Nilsson, 2001).

Properties related to soil organic matter, on one hand, and to parent material on the other, may also be important in microbial community functioning. Previous studies have indicated that soil organic matter mediates microbial substrate utilization in arable (Degens et al., 2000) and grassland soils (Grayston et al., 2003) and the present results indicate that this could be extended to forest soils. Indeed, we found that litter mass, soil organic C, total N and available P were all correlated with CLPP in these temperate forests. Water holding capacity also strongly influenced the functioning of the microbial community (Drenovsky et al., 2004). Furthermore, variations in microbial activity were clearly related to the concentrations of calcium in both forest floor and soil. Lamarche et al. (2007) also concluded that geological parent material exerts an indirect but strong influence on microbial community, possibly by modifying the base cation status and pH of plant litter.

6.5. Conclusions

In conclusion, subtle differences in litter quality were observed in *Quercus robur*, *Quercus ilex*, *Fagus sylvatica* and *Pinus radiata* at the landscape-level in the temperate humid climate region under study. However, functionally distinct microbial communities were observed in the ecosystems under study, which supports the idea that community level physiological profiles (CLPP) of microbial community constructed with MicroResp^{TM} could be used to differentiate among temperate forest types at this landscape-level and consequently, that organic matter dynamics may differ in these forests. Finally, we cannot consistently support with our data the hypothesis that plant community structure is the major determinant of soil microbial community function. Evidence of the influence of soil organic matter and parent material on CLPP was found in this study. We therefore conclude that differences in CLPP probably result from complex

interactions between soil properties that mediate substrate availability, abiotic factors that mediate cation base status, and vegetation type, which mediates substrate quality.

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7. Conclusiones Generales

- La dinámica y estructura de la MO del mantillo es diferente bajo cubiertas forestales de roble, haya o pino bajo condiciones climáticas, geomorfológicas y edáficas similares. La tasa de descomposición más lenta se observa en el pinar maduro debido principalmente a la calidad del matillo (pH más acido, ratios de C/N y lignina/N más elevados y mayor concentración de grupos fenólicos en el horizonte FH). El robledal, con la ausencia total del horizonte FH del mantillo presenta la tasa de descomposición más rápida mientras que el hayedo presenta una tasa de descomposición intermedia. La diferencia en la dinámica de la MO en los bosques seminaturales se debe principalmente a diferencias en la comunidad microbiana. El hayedo con un porcentaje de humedad más elevado podría tener un ratio de bacteria/hongos más elevado que el robledal, influyendo de esta manera en la dinámica de la MO.
- El horizonte superficial del suelo (0-5 cm) se percibe más sensible que el subsuperficial (5-15 cm) para detectar los cambios producidos en el funcionamiento del mismo por la intensificación de la gestión forestal.
- El suelo del pinar maduro presenta valores muy elevados de actividad de fosfatasa ácida y un alto porcentaje del fosfolípido 18:2ω6 (indicador fúngico) frente al total, reflejando el importante estrés nutritivo de fósoforo de los pinares.
- El conjunto mínimo de parámetros que mejor describe los cambios producidos en el suelo de los sistemas forestales estudiados son: físicos como el contenido de agua a capacidad de campo (-33KPa) y el contenido de agua cuando el 10% de la porosidad del suelo está llena de aire; químicos como pH y la concentración de fósforo disponible; y biológicos como la relación entre bacterias Gram+ y Gram- .
- El diámetro medio de agregado (MWD) es mayor en los rodales seminaturales (roble y haya) que en el pinar maduro.
- La teoría jerarquica de Tisdall y Oades (1982) no se cumple en estas parcelas forestales y la relación de C-POM/C-total con el MWD nos hace concluir que en este tipo de sistemas la fracción POM juega un papel de primer orden en la agregación.
- Las diferencias observadas en el estudio particular del mantillo se disipan cuando analizamos los mantillos sin separación de horizontes y escala de paisaje.
- MicroResp^{TM} parece una metodología adecuada y prometedora para el estudio de la comunidad microbiana a dicha escala.

EFECTO DE TÉCNICAS DE PREPARACIÓN DE SUELO MECANIZADAS SOBRE LA ESTRUCTURA Y DINÁMICA DE LA MO:

- El establecimiento de plantaciones forestales de pino mediante mecanización intensiva hace desaparecer la totalidad del mantillo del suelo forestal. La cantidad de mantillo es todavía un 50% menor que en un pinar maduro 16 años después de la mecanización. Además, la tasa de descomposición de este pinar es mayor que la del maduro y su comunidad microbiana presenta un índice de diversidad funcional menor.
- El efecto de la mecanización sobre la física del suelo queda reflejada en una gran disminución del LLWR (Least Limiting Water Range) debido al incremento de la densidad aparente. Dieciseis años después de la mecanización este indicador de la perturbación física (LLWR) sigue siendo menor que en el pinar maduro. El LLWR se muestra como un indicador más robusto frente a las pertubaciones derivadas de la mecanización que el agua útil.
- La mecanización disminuye drásticamente el diámetro medio de agregado (MWD). Este parámetro parece recuperarse tras 16 años.
- La mecanización disminuye la cantidad de la fracción más lábil de MO (C-POM) que no se ha recupera tras 16 años y su calidad sigue siendo diferente (C/N-POM). Dicha fracción lábil se correlaciona positivamente con la respiración microbiana y la mineralización de nitrógeno, por lo que el efecto de la mecanización en este reservorio puede tener consecuencias en la fertlidad a largo plazo en las plantaciones forestales mecanizadas.

EFFECT OF SPECIES CHANGE ON ORGANIC MATTER STRUCTURE AND DYNAMICS IN FOREST STANDS:

- The dynamics and structure of forest floor organic matter differ in oak, beech and pine stands under similar climatic, geomorphological and edaphic conditions. The slowest rate of decomposition was observed in the mature pine stand, mainly because of the quality of the humus (more acidic, higher ratios of C/N and lignin/N and higher concentrations of phenolic groups in the FH horizon than in the other stands). The fastest rate of decomposition was observed in the oak stand, in which there was no FH horizon. An intermediate rate of decomposition was observed in the beech stand. The difference in the OM dynamics in semi-natural forests is mainly due to differences in the microbial community. The moisture content of the soil under the beech stand was high and therefore the ratio of bacteria/fungi may be higher than in the oak, which would therefore affect the OM dynamics.
- The surface horizon of the soil (0-5 cm) was more sensitive than the subsurface horizon (5-15 cm) in terms of indicating the changes produced in the functioning by the management techniques applied.
- High values of acid phosphatase activity and a high percentage of phospholipid 18:2ω6 (a fungal indicator) were observed in the soil under the mature pine, reflecting the high degree of nutritive stress on phosphorus in pine stands.
- The minimum set of parameters that best describes the changes produced in the soil in the forest systems studied were: physical, such as the water content at field capacity (-33KPa) and the water content when 10% of the soil pores are filled with air; chemical, such as pH and the concentration of available phosphorus, and biological, such as the ratio between Gram +ve and Gram-ve bacteria.
- The mean weight diameter (MWD) of the aggregate is higher in the semi-natural stands (oak and beech) than in the mature pine.
- The hierarchical theory of Tisdall and Oades (1982) does not hold in the forest stands under study and the relation between POM-C/ total-C and the MWD allows us to conclude that the POM fraction plays a major role in aggregation.
- The differences observed in the study, particularly as regards the forest floor layer, disappeared when the forest floor layers were analysed without separating the horizons, and at landscape scale.
- MicroResp^{TM} appears to be an adequate and promising method for the study of the microbial community at the landscape scale.

EFFECT OF MECHANIZED SOIL PREPARATION TECHNIQUES ON THE ORGANIC MATTER STRUCTURE AND DYNAMICS:

- The establishment of pine stands by intensive, mechanized techniques results in total loss of the forest floor layer of the forest soil. The quantity of the forest floor layer was still 50% less than in a mature pine stand, 16 years after the mechanized treatment. In addition, the rate of decomposition in this stand was greater than in the mature stand and the functional diversity index of the microbial community was lower.
- The effects of mechanized treatments on the physical aspects of the soil were reflected in the large decrease in the LLWR (Least Limiting Water Range) due to the increase in apparent density. Sixteen years after the mechanized treatment, this indicator of physical disturbance (LLWR) was still lower than in the mature pine. The LLWR was observed to be a more robust indicator of the disturbances derived from the mechanized treatments than the available water.
- The mechanized treatments resulted in a drastic decrease in the mean weight diameter (MWD) of the aggregates. The parameter appeared to have recovered 16 years after stand establishment.
- The mechanized treatments resulted in a decrease in the amount of the most labile fraction of the OM (POM-C), which had not recovered 16 years after establishment and the quality still differed (POM-C/ POM-N). The labile fraction was positively correlated with microbial respiration and nitrogen mineralization, and therefore the effect of mechanization on this reservoir may have consequences for the long term fertility of the soils in mechanized forest stands.

ESPEZIE ALDAKETAREN ERAGINA MATERIA ORGANIKOAREN EGITURA ETA DINAMIKAN BASO-EKOSISTEMETAN:

- Orbelaren egitura eta dinamika ezberdinak dira haritz, pago edo pinupean, nahiz eta baldintza klimatiko, geomorfologiko eta edafiko antzekoetan garatu. Materia organikoaren deskonposizio tasarik geldoena pinudian ematen da, batik bat orbelaren kalitatea dela eta (pH azidoagoa, C/N eta lignina/N erlazio altuagoa eta konposatu fenolikoen kontzentrazio altuagoa FH horizontean). Hariztiak, FH horizonterik gabe, deskonposizio tasarik bizkorrena du eta pagadiak tarteko deskonposizio abiadura erakutsi du. Harizti eta pagadiaren arteko deskonposizio tasa ezberdintasuna orbeleko mikrobio-komunitatearen araberako da. Pagadiak orbel hezeagoa du eta horrek, hariztiak baino bakterio/onddo erlazio altuagoa izatea suposa lezake, materia organikoaren dinamikan eraginez.
- Lurzoruko goi horizontea (0-5 cm) baso-jardueren areagotzeak beronen funtzionamenduan eragiten dituen aldaketak hautemateko sentikorragoa da lurzoruko behe horizontea (5-15 cm) baino.
- Pinudiko lurzoruak fosfatasa azidoaren eta 18:2ω6 fosfolipidoaren (onddoen indikatzailea) balio oso altuak erakusten ditu, pinudiek fosforo eskasia dutela bistaratuz.
- Ikertutako baso-ekosistema epeletan, baso-jardueren eraginez lurzoruan sortutako aldaketak hoberen deskribatzen duen gutxieneko parametro multzoa honakoa da: parametro fisikoak, lurzoruaren ur edukiera eremu kapazitatean (-33KPa) eta poroen %10 airez beterik dagoeneakoa; parametro kimikoak, pH eta fosforo erabilgarria; eta parametro biologikoak, bakterio Gram-positibo eta Gram-negatiboen arteko erlazioa, adibidez.
- Lurzoruko agregatuen batazbesteko diametroa (MWD) handiagoa da harizti eta pagadian pinudian baino.
- Tisdall eta Oades (1982)-en agregatuen sorkuntzarako teoria hierarkikoa ez da betetzen ikertutako baso-ekosistemetan. Baso hauetan materia organikoaren frakzioa labilak agregatuen sorkuntzan papel berezia betetzen duela ondorioztatzen da, C-POM/C-total eta MWD-ren arteko erlazio sendotik.
- Orbelaren ikerketa zehatzean espezie ezberdinen artean behatutako orbelaren ezaugarrietan diferentziak desagertu egiten dira ezaugarri hauek paisaia eskala batean neurtzen badira eta orbela ez bada horizonte ezberdinetan banatzen.
- MicroResp^{TM} metodologia egokia eta etorkizun handikoa da lurzoruko mikrobio komunitatearen azterketak paisaia eskalan garatzeko.

LURZORU PRESTAKUNTZA MEKANIZATUEN ERAGINA MATERIA ORGANIKOAREN EGITURA ETA DINAMIKAN BASO-EKOSISTEMETAN:

- Baso-landaketak ezartzeko erabilitako baso-jarduera mekanizatuek orbela bere osotasunean desagertarazten dute eta pinudi heldu batekin alderatuz, oraindik %50 baxuagoa da mekanizazioa gertatu eta 16 urte beranduago. Gainera, orbelaren deskonposizio tasa altuagoa eta mikrobio komunitatearen dibertsitate funtzionalaren indizea baxuagoa dira 16 urteko pinudi honetan pinudi helduan baino.
- Mekanizazioaren eragina lurzoruko egitura fisikoan LLWR (Least Limiting Water Range) parametro konposatuaren itzelezko murrizketan islatu da, gehienbat itxurazko dentsitatearen areagotzearen ondorioz. Mekanizazioa gertatu eta hamasei urte beranduago, perturbazio fisikoaren indikatzaile hau (LLWR) oraindik ere pinudi helduan baino baxuagoa da. LLWR, mekanizazioak ur erabilgarriaren gainean duen eragina aztertzeko indikatzaile egokia da.
- Lurzoru prestakuntza mekanizatuak lurzoruko agregatuen batazbesteko diametroa (MWD) izugarri txikitzen du, nahiz eta parametro hau 16 urteren buruan berreskuratu egiten den.
- Mekanizazioak lurzoruko materia organikoaren frakzio labila murrizten du (C-POM) eta frakzio hau ez da berreskuratzen 16 urteren buruan ez kantitatean ez kalitatean. Gainera, frakzio labil hau lurzoruko mikrobio biomasaren arnasketarekin eta nitrogeno mineralizazioarekin positiboki erlazionatzen da, beraz, mekanizazioak materia organikoaren erreserba honetan izan dezakeen eraginak baso-landeketen epe luzeko emankortasunean ondorio zuzena izan dezake.

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