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Valorisation d'un compost de boues urbaines en garrigue pour le reboisement : comportement des jeunes arbres d'une plantation et modifications de la dynamique de la végétation naturelle apres amendement

Marie Guittonny - Larcheveque

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Marie Guittonny - Larcheveque. Valorisation d'un compost de boues urbaines en garrigue pour le reboisement : comportement des jeunes arbres d'une plantation et modifications de la dynamique de la végétation naturelle apres amendement. *Ecologie, Environnement*. Université de Provence - Aix-Marseille I, 2004. Français. NNT: . tel-00008812

HAL Id: tel-00008812

<https://theses.hal.science/tel-00008812v1>

Submitted on 17 Mar 2005

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REMERCIEMENTS

En tout premier lieu, je tiens à remercier le professeur Gilles Bonin pour sa confiance, sa disponibilité constante tout au long de mon travail de thèse, ainsi que pour ses incitations à pousser plus loin les investigations vers la conceptualisation. J'ai beaucoup appris à son contact.

Je suis honorée que les professeurs Thierry Gauquelin et Michèle Trémolières aient accepté d'être rapporteurs de ma thèse et je les en remercie vivement.

C'est avec grand plaisir que je remercie Jean-Michel Carnus et Ingrid Walter pour leur participation au jury.

La bouillonnante personnalité de Nicolas Montès, ainsi que sa gentillesse, sa compétence et son exigence ont permis à mon travail de thèse d'avoir une saveur joyeuse et sérieuse en même temps. De plus, mon codirecteur de thèse fût d'une grande aide pour l'incorporation de photos et figures à base d'images dans cette thèse. Pour tout cela, merci, et aussi pour sa patience dans nos discussions « animées » !

La femme que je remercie ici est Christine Ballini qui a été mon encadrante lors de mon stage de DEA au LBEM – travail préparatoire à celui-ci – mais qui fut aussi une « codirectrice officielle » de ce travail, par sa générosité à s'impliquer autant dans le travail sur le terrain que lors de la mise en forme écrite de ce travail.

Ca commence par une blague, et ça finit dans un éclat de rire ! Merci à Virginie Baldy pour cette étonnante faculté à travailler rigoureusement dans une ambiance détendue. De l'ergostérol aux comptages bactériens, je garde en mémoire la disponibilité et l'exigence scientifique qui sont les siennes. Merci de m'avoir introduit au sein du laboratoire d'Eric Chauvet à Toulouse, où j'ai bénéficié d'un accueil très chaleureux.

Merci à Catherine Fernandez pour sa gentillesse et sa constante disponibilité, notamment pour faire partager ses connaissances en statistiques.

Je remercie Nathalie Korboulewsky de sa vivacité et de son implication dans la rédaction scientifique.

Je remercie les membres du LBEM pour leur présence sur le terrain, qui a permis d'abattre un travail considérable, surtout Sylvie Dupouyet (à qui le mistral ne fait pas peur !).

Sans oublier Stéphane Greff, qui m'a énormément apporté dans le domaine des analyses chimiques par son exigence constante, sa grande capacité au travail, et son humeur toujours égale. Un immense merci !

J'ai beaucoup apprécié les échanges conceptuels et/ou philosophiques avec Mathieu Planquette, ainsi que la bonne humeur d'Elena Ormeño. Merci à tous deux !

REMERCIEMENTS

Cette étude a bénéficié d'une étroite collaboration avec la Direction de l'Environnement du Conseil Général des Bouches du Rhône. Je tiens à remercier les membres du Bureau de l'Eau, passés ou présents, qui ont initié la mise en place de l'étude (Jean-Marc Philip, Béatrice Orelle) et l'ont suivie avec compétence (Benjamin Durand), ainsi que Romuald Budet et Olivier Dubout pour leur gentillesse et leur disponibilité. Je leur souhaite une excellente continuation sur le sujet, en collaboration avec le service Traitement des Déchets. Je souhaite également remercier la Direction de la Recherche du Conseil Général, qui a financé l'achat d'une Chromatographie ionique, et m'a ainsi permis d'effectuer les analyses du contenu nutritionnel des végétaux.

Merci également aux membres de la Garde à cheval pour les cafés matinaux et la glace à la banane !

Je souhaite remercier l'ADEME et la Direction de l'Environnement et de l'Energie du Conseil régional PACA pour leur soutien financier qui a permis la réalisation de ce travail. Merci à Béatrice Müller pour l'intérêt qu'elle a porté à cette étude en tant que tutrice de l'ADEME, ainsi qu'à Mr Jacques Fiol, pour son appui dans l'obtention de ma bourse de thèse.

Je tiens à remercier les membres du comité de suivi de l'expérimentation pour leurs remarques constructives et leur intérêt constant, notamment Rémi Mouton de la Mission de Recyclage Agricole des Déchets de la Chambre d'Agriculture des Bouches du Rhône.

Merci à l'entreprise Biotechna qui a bien voulu fournir le compost nécessaire aux expérimentations, et financer la valorisation d'une partie des résultats obtenus au colloque *Waste Management 2004*.

Je remercie les amis qui m'ont soutenue et avec qui j'ai passé de très bons moments durant ces trois années, Elise, Fatiha, Jean et Philou.

Enfin, je remercie mon entourage proche pour leur soutien et leur chaleureuse attention, qui m'ont beaucoup aidé durant ces trois années, notamment mes parents et beaux parents.

A toi Philippe, qui illumine ma vie depuis deux ans et demi, a partagé les moments difficiles comme ceux d'intense bonheur, et a fait preuve d'une patience d'ange, je réserve cet espace particulier.

Pour tout cet amour et cette tendresse partagés, Merci.

A charge de revanche pour les deux années qui viennent !

RESUME

Depuis que la Communauté européenne a interdit la mise en décharge des déchets non ultimes, la recherche de nouveaux débouchés pour les boues d'épuration devient cruciale. En région méditerranéenne, les feux récurrents et les précipitations parfois violentes favorisent l'érosion et induisent l'appauvrissement des sols. Les sols calcaires dégradés sont souvent colonisés par des formations végétales stables à *Quercus coccifera*, adaptées à un faible niveau de ressources, ce qui ralentit l'installation naturelle des arbres. Dans de tels milieux, un amendement organique pourrait améliorer la fertilité du sol, modifier la dynamique et les relations de dominance de la végétation, et favoriser les processus de succession et de résilience, ainsi que la reforestation.

Deux expérimentations ont été menées en Provence pour étudier les effets d'apports de compost de boues d'épuration urbaines et de déchets verts sur la dynamique d'un écosystème de garrigue. Premièrement, 0, 50, et 100 t.ha⁻¹ de compost brut ont été épandues en surface dans le biotope naturel de garrigue, et les conséquences sur le fonctionnement de l'écosystème ont été étudiées. Les compartiments sol, végétation et microorganismes de la litière ont été suivis pendant deux ans après amendement (2002-2004). Deuxièmement, nous avons incorporé au sol trois doses de compost brut (0, 20, et 40 kg.ha⁻¹) au pied de jeunes plants d'arbres. L'évolution des propriétés édaphiques et du développement des arbres a été suivie respectivement pendant trois ans (2001-2003) et deux ans (2002-2003).

L'amendement a durablement (2 ans) et efficacement amélioré la fertilité du sol. Cependant, le sol a été fortement enrichi en P et Zn, ce qui pourrait entraîner des problèmes sérieux de pollution des eaux et de toxicité sur les microorganismes. Ces deux éléments limitent l'utilisation de compost de boues dans les milieux naturels, et des amendements répétés aux doses étudiées sont inenvisageables.

Dans la plantation, l'amendement a globalement favorisé le processus de reforestation en améliorant la nutrition et la croissance des plants, et surtout en augmentant leur survie en période de sécheresse. En outre, l'apport de compost en surface a généré l'apparition d'îlots fertiles dans le biotope garrigue, au sein desquels les processus de succession végétale pourraient être localement optimisés, ce qui pourrait favoriser à terme la colonisation par la strate arborée, notamment par *Pinus halepensis*. Par ailleurs, l'amendement augmente la diversité fonctionnelle en garrigue du fait de son effet bénéfique sur les espèces semencières, ce qui pourrait améliorer la résilience de l'écosystème après incendie.

Ces effets bénéfiques du compost se produisent surtout pour les doses intermédiaires. L'utilisation de compost aux doses maximales testées devrait être abandonnée, du fait d'une dépréciation de la colonisation fongique de la litière de *Quercus coccifera* observée pour 100t.ha⁻¹, et du fait d'une augmentation des risques de pollution par le P et le Zn proportionnellement au taux d'apport. Cependant, la nature calcaire du sol expérimental et la maturité élevée du compost ont limité les phénomènes d'exportation vers les eaux et la contamination des plantes par les éléments potentiellement toxiques. L'apport de compost en surface a plutôt augmenté la sensibilité des plantes à la sécheresse, alors que celle-ci a diminué lorsque le compost a été apporté en mélange au sol.

Enfin, l'apport de compost en garrigue a eu des effets multiples (fertilisation, écrasement, dépôt d'une couche fertile) et a permis de mettre en évidence les stratégies spécifiques des plantes dans la gestion des nutriments et de la productivité. Ce travail montre l'importance relative des espèces végétales sur le fonctionnement de l'écosystème, identifie les espèces clef pour le recyclage des éléments, et illustre la complémentarité d'utilisation des ressources qui existe au sein de l'écosystème naturel de garrigue.

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INTRODUCTION GENERALE

Chapitre 1

CONTEXTE DE L'ETUDE ET PROBLEMATIQUE

1. Le contexte écologique méditerranéen

Le climat méditerranéen est caractérisé par une sécheresse estivale (Bagnouls & Gaussen 1957). Celle-ci, associée aux vents violents qui balayent la zone méditerranéenne, favorise les incendies récurrents. (Scarascia-Mugnozza *et al.* 2000 ; De Luis *et al.* 2001). L'alternance de saisons sèches et pluvieuses, ces dernières étant caractérisées par des pluies souvent violentes (Le Houerou 1973 ; Scarascia-Mugnozza *et al.* 2000), ainsi que l'élimination du couvert végétal par les feux prédisposent les sols méditerranéens à l'érosion. De plus, le feu consomme la matière organique du système sol/plante (végétation aérienne, litière, matière organique des couches superficielles du sol) et minéralise le stock de nutriments qu'elle contient. Ces nutriments sont soit volatilisés (N, Blair 1997), soit se retrouvent dans les cendres déposées sur le sol et sont alors facilement exportés par ruissellement (Grogan *et al.* 2000). Ainsi, incendies récurrents et érosion induisent un appauvrissement permanent des sols méditerranéens en matière organique et en nutriments (Le Houerou 1973).

1.1 La végétation méditerranéenne

1.2.1 Caractéristiques fonctionnelles

Le climat, les incendies fréquents, ainsi que l'infertilité des sols ont favorisé le développement d'une végétation soumise à des perturbations récurrentes et adaptée au stress hydrique, ainsi qu'à de faibles niveaux de ressources édaphiques. Les écosystèmes méditerranéens sont dominés par des espèces végétales sclérophylles d'une part, dont les feuilles lignifiées ont des pertes en eau faibles, et sempervirentes d'autre part, ce qui leur permet de prolonger l'activité photosynthétique au-delà de la saison estivale (Rundel 1988 ; Archibold 1995). Par ailleurs, la récurrence des feux a induit la dominance d'espèces pyrophytes, dont la multiplication et / ou la reproduction sont stimulées par le passage du feu (Le Houerou 1973). Ces végétaux peuvent rejeter de souche après destruction d'une partie ou de la totalité de leur biomasse aérienne (Baeza *et al.* 2003), ou bien régénérer par germination à partir de la banque de graines du sol (reproduction sexuée) (Calvo *et al.* 2002^a). Enfin, les végétaux sempervirents développent des stratégies qui limitent leurs pertes en éléments

nutritifs et qui constituent une adaptation à la faible disponibilité des ressources édaphiques. Ainsi, la longue durée de vie des tissus foliaires sclérophylles, la fabrication préférentielle de tissus pérennes et peu concentrés en nutriments (tiges et racines), ainsi que des taux de retranslocation des éléments élevés avant sénescence limitent la restitution des nutriments au sol (Rundel 1988 ; Aerts 1995).

1.2.2 Dynamique de la végétation en Provence calcaire

Ecosystèmes forestiers

Les trois principales espèces arborées en Provence calcaire appartiennent à des modèles théoriques d'occupation spatiale différents (Barbero *et al.* 1990^b) : *Pinus halepensis* (expansionniste), *Quercus ilex* (résistance) et *Quercus pubescens* (stabilisation). La plasticité écologique du conifère *Pinus halepensis* lui permet d'être présent sous des contraintes trophiques et des stress climatiques variés. De plus, la bonne survie des graines dans différents types de milieux et leur grande capacité de dispersion par le vent confère à cette espèce une grande amplitude spatiale. *Quercus ilex*, feuillu sclérophylle rejetant de souche, montre une forte amplitude écologique, une forte plasticité climatique, ainsi qu'un bon ajustement au stress hydrique estival. Enfin, *Quercus pubescens* investit dans la longévité et une production de biomasse continue, étalée sur plusieurs siècles. Cette espèce est peu adaptée à des cycles de perturbations réguliers, à de fortes fréquences et intensités, ce qui limite sa présence dans les zones exposées au feu.

Dégradation et apparition de garrigues

L'augmentation de la fréquence des perturbations liée à l'anthropisation croissante favorise l'apparition de séries régressives sur les zones les plus dégradées, où la végétation arborée est remplacée par une végétation pyrophyte adaptée aux feux récurrents (Le Houerou 1973 ; Barbero 1990^a). Ces séries régressives sont caractérisées par des peuplements de plus en plus appauvris en biodiversité après chaque perturbation (Ramade 2002). Après chaque feu, les espèces principales de la communauté végétale préexistante se réinstallent par un processus d'autosuccession (Ferran & Vallejo 1998), et en conséquence, ces communautés végétales présentent une relative stabilité écologique et floristique (Lepart & Escarré 1983).

En Provence calcaire, les zones les plus dégradées sont colonisées par des garrigues à *Quercus coccifera* (Barbero 1990^a), définies comme des forêts dégradées à végétation basse et xérophile plus ou moins ouverte (Le Houerou 1971), et considérées par certains auteurs comme un stade de blocage de la succession végétale (Lepart & Escarré 1983 ; Cañellas &

San Miguel 1998 ; Quézel & Médail 2003). Cette espèce sclérophylle et sempervirente forme des communautés relativement stables du fait de sa durée de vie importante et du rajeunissement continu de sa partie aérienne par rejet de souche après incendie (Keeley 1986). De plus, elle occupe rapidement l'espace ouvert après incendie et diminue ainsi l'espace disponible pour les autres espèces (Lepart & Escarré 1983).

2. Niveau de ressources des écosystèmes et dynamique de la végétation

Les espèces appartenant à un écosystème sont pour la plupart inféodées à des caractéristiques environnementales données auxquelles elles sont bien adaptées (Schulze 1995). La disponibilité en azote et autres nutriments est un facteur environnemental déterminant pour la dynamique des communautés végétales dans de nombreux écosystèmes (Berendse 1994 ; Berendse *et al.* 1998). En effet, la dynamique des écosystèmes repose sur des relations étroites entre sol et végétaux, qui se traduisent par des flux continus de matière, d'éléments chimiques et d'énergie (Gimeno-Garcia *et al.* 2001). Des modifications dans la disponibilité des nutriments peuvent fortement affecter la compétition entre espèces et la composition des communautés végétales (Tilman 1984).

La végétation de garrigue, dominée par *Quercus coccifera*, est en équilibre avec le milieu édaphique pauvre en matière organique et en nutriments, dans lequel elle est très compétitive. D'après Gosz (1984), dans des milieux où un ou plusieurs nutriments sont limitants, augmenter leur disponibilité peut induire une plus grande absorption des éléments et une plus grande fixation du carbone par les plantes, ce qui améliore la qualité de la litière produite, accroît les taux de décomposition et entretient la plus forte disponibilité en nutriments. Or, des expériences ont montré que les habitats riches en nutriments sont généralement dominés par des espèces avec un taux de croissance élevé (Aerts & Van Der Peijl 1993 ; Urbas & Zobel 2000). Ainsi, une augmentation du niveau de ressources peut entraîner le remplacement des espèces végétales à faible taux de croissance et faibles pertes de biomasse par des espèces à fort taux de croissance et pertes de biomasse élevées (Berendse 1998).

D'autre part, la végétation de garrigue à *Quercus coccifera*, qui s'installe sur les sols dégradés par le passage répété du feu, constitue une phase de blocage de l'évolution vers la strate arborée. La dominance d'espèces qui rejettent de souche telles que *Q. coccifera* et dans une moindre mesure *Brachypodium retusum*, qui occupent rapidement l'espace par

reproduction végétative après le passage du feu et empêchent l'installation d'autres espèces, induit une diminution de la diversité génétique, ainsi que de la diversité fonctionnelle et floristique de l'écosystème. La banque de graines s'appauvrit après chaque feu. De plus, il semble que la capacité à rejeter de souche diminue au cours d'incendies successifs (Diaz-Delgado *et al.* 2002). En conséquence, la capacité de régénération après perturbation diminue et l'évolution de l'écosystème vers le stade forestier potentiel est retardée. Par ailleurs, *Quercus coccifera* entretient le faible niveau de ressources du milieu garrigue (Aerts 1995) par une production graduelle de litière peu décomposable, qui induit une restitution très progressive des nutriments au sol et ralentit les cycles des éléments (Rundel 1988).

3. Le contexte des boues d'épuration

3.1 Définition et production

Les boues de stations d'épuration sont constituées des sédiments résiduaux provenant du traitement des eaux usées. Depuis le 8 décembre 1997 (décret n°97-1133), elles sont considérées comme un déchet, défini juridiquement comme tout résidu d'un processus de production, de transformation, ou d'utilisation, toute substance, matériau, produit que son détenteur destine à l'abandon (loi du 15/07/1975 relative à l'élimination des déchets et à la récupération des matériaux). En France, l'épuration des eaux usées urbaines produit 1 million de tonnes de matière sèche de boues par an (Michaut 2004), et il est prévu une production de 1,3 million en 2005. La quantité de boues produites augmente chaque année. En effet, l'Union Européenne impose que toute ville de plus de 2000 équivalent habitant épure ses eaux usées à l'horizon 2005 (directive 91-271). En outre, l'amélioration des conditions de branchement des usagers domestiques d'une part, de la qualité des réseaux d'assainissement et du rendement des stations d'épuration d'autre part, induisent également un accroissement de la production de boues (Courtois 2000).

3.2 Voies d'élimination

Actuellement en France, 60 % des boues produites sont recyclées en agriculture (sur moins de 1 % de la surface agricole utile), 20 à 25 % sont mises en décharge et 15 à 20 % incinérées (Courtois 2000). En effet, les boues d'épuration sont riches en carbone organique et en éléments fertilisants (N, P, K), ce qui leur confère un intérêt agronomique et explique leur utilisation en agriculture. De plus, ce sont des amendements peu chers pour les agriculteurs. Cependant, elles contiennent également des éléments traces métalliques (ETM) et organiques

(ETO : hydrocarbures aromatiques polycycliques, composés organochlorés), des molécules issues des traitements médicaux humains, ainsi que des organismes pathogènes (bactéries, virus, amibes, vers plats), ce qui peut poser des problèmes de contamination des sols et des cultures. De la même manière, ces éléments potentiellement toxiques, ainsi que les nitrates et phosphates apportés par les boues, peuvent être transférés vers les eaux souterraines et de surface après épandage et induire la pollution des nappes phréatiques et l'eutrophisation des cours d'eau. Cela explique la réticence de certains agriculteurs à utiliser les boues d'épuration comme amendement.

Cependant, la mise en décharge et l'incinération semblent mal adaptées à l'élimination des boues d'épuration. En effet, depuis le 1^{er} juillet 2002, l'application de la loi européenne du 13 juillet 1992 supprime les décharges brutes, afin d'autoriser la seule mise en décharge des résidus ultimes, c'est à dire des déchets qui ne sont plus susceptibles d'être traités ou transformés, notamment par extraction de la part valorisable ou par réduction de leur caractère polluant ou dangereux. Par ailleurs, l'incinération des boues brutes, déchets très riches en eau, est peu rentable car elle implique une déshydratation préalable par filtration, centrifugation ou séchage, facilitant leur transport et permettant un meilleur rendement.

3.3 Epandage et réglementation

En conséquence, les instances décisionnelles, conscientes que de nos jours le problème de l'accumulation des résidus pose celui de l'épuisement des gisements de matières premières, ont autorisé l'épandage des boues en agriculture, tout en mettant en place une réglementation stricte visant à minimiser la contamination des sols agricoles. Le sol est considéré ici comme un système tampon naturel, un filtre régulant le transport d'éléments et de substances chimiques vers l'atmosphère, l'hydrosphère et les êtres vivants (Zakoski & Edmonds 1986 ; Kabata-Pendias & Pendias 1992). Cependant, le rôle le plus important du sol étant lié à sa productivité, essentielle pour la survie des humains, le maintien de ses fonctions écologiques et agricoles est de première importance.

Ainsi, le décret n°97-1133 stipule que tout épandage est soumis à la loi sur l'eau (3 janvier 1992), et rend obligatoire d'une part la réalisation d'une étude préalable (sol et boues) aboutissant à un plan d'épandage, et d'autre part la surveillance qualitative des flux d'éléments potentiellement contaminants après épandage. L'arrêté du 8 janvier 1998 définit le contenu de l'étude préalable, les conditions de stockage des boues et le suivi après épandage. De plus, il limite l'apport de boues à 30 t.ha⁻¹ de matière sèche sur une période de 10 ans et sur des sols dont le pH est supérieur à 6. Enfin, il fixe les teneurs maximales

admissibles en éléments indésirables dans les boues et les sols avant épandage, leur flux maximal sur 10 ans, ainsi que les fréquences et les méthodes d'analyse.

3.4 Transformation des boues et normalisation

Il existe un 2^{ème} statut législatif pour les boues d'épuration valorisées en agriculture. En effet, lorsque celles-ci sont transformées ou bien entrent dans la composition d'amendements, engrais ou supports de culture, elles peuvent passer du statut de déchet à celui de produit commercialisé. Elles relèvent alors de la réglementation sur les matières fertilisantes et le produit final doit soit faire l'objet d'une homologation, soit être conforme à une norme d'application obligatoire (Feix & Savanne 1998). La normalisation concerne des familles de produits répondant à des critères d'innocuité et de qualité concernant la présence de pathogènes, d'éléments traces métalliques, d'éléments traces organiques et de contenu fertilisant. Ainsi, la norme NF U 44-095 a été créée en mai 2002 par l'Agence française de normalisation (Afnor) et concerne les composts contenant des Matières d'Intérêt Agronomique issues du Traitement des Eaux (M.I.A.T.E.), dont les boues d'épuration urbaines. Elle a été mise en application par l'arrêté publié au Journal Officiel du 26 mars 2004.

3.5 Compostage : hygiénisation et stabilisation

Le compostage est un procédé biologique contrôlé de conversion et de valorisation des substrats organiques, sous produits de la biomasse et déchets organiques d'origine biologique, en un produit stabilisé, hygiénisé, semblable à un terreau, riche en composés humiques (Faurie *et al.* 2003).

- Ce procédé comporte une première phase de dégradation (environ 1 mois), accompagnée d'une élévation de température qui implique une succession de microorganismes plus ou moins thermophiles. Durant cette étape, où la température atteint jusqu'à 70 °C, est réalisée l'élimination des organismes pathogènes des boues.

- La deuxième phase de maturation, plus ou moins longue, consiste en une stabilisation progressive de la matière organique (Bernal *et al.* 1998). L'humification plus ou moins poussée, fonction de la durée de maturation, permet un relargage progressif des éléments nutritifs du compost, diminuant les risques de lessivage et de contamination des eaux. Ainsi, Epstein *et al.* (1978) notent que la minéralisation de l'azote apporté lors d'un épandage de boues compostées est lente et proche de celle d'un sol sans amendement.

- La troisième et dernière phase est le criblage (tamisage à 4 cm), qui permet de retirer du compost les produits grossiers non dégradés. En conséquence, le compostage permet d'obtenir un produit hygiénisé et stabilisé par rapport aux boues d'épuration entrant dans sa composition.

4. Problématique

L'augmentation incessante de la production de boues, ainsi que l'interdiction de leur mise en décharge implique de leur trouver de nouvelles voies de recyclage. La transformation des boues en compost conforme à la norme NF U 44-095 ouvre de nouvelles perspectives. Elle pourrait augmenter leur recyclage en agriculture. En effet, les boues de stations d'épuration ne représentent que 1,7 % des déchets épandus en agriculture chaque année (ADEME 1999). Cependant, d'autres voies de recyclage des boues compostées peuvent constituer une alternative à l'épandage en agriculture. Ainsi, la revégétalisation des bordures d'autoroutes, des anciennes carrières et des pistes de ski se développe de plus en plus. De même, l'épandage de compost de boues en forêt, pour la reforestation ou la restauration de milieux dégradés peut être envisagé. En 2001, la ville de Marseille dirigeait encore 50 % de ses boues d'épuration vers les décharges (Le Scaon 2001). Dans ce contexte local, il semble urgent d'expérimenter de nouvelles voies de recyclage de ces boues.

L'utilisation de compost de boues d'épuration pour restaurer des sols méditerranéens dégradés par le passage répété du feu pourrait modifier le fonctionnement du système sol / plantes en agissant sur les transferts d'éléments, et induire une réorganisation des relations entre espèces végétales plus propice à l'évolution vers les stades arborés potentiels.

L'objet de ce travail est de déterminer quels vont être les changements induits par un apport de compost en garrigue :

- sur le recyclage des éléments du sol par la végétation et les organismes décomposeurs
- sur la diversité fonctionnelle de la végétation
- sur l'installation des espèces arborées naturelles.

Les éventuelles conséquences sur les capacités de résilience après incendie, et sur les processus successionnels seront évoquées. En outre, la contamination du milieu naturel par les éléments trace métalliques, et par les sels apportés (notamment les formes de phosphore)

par un compost humifié et peu contaminé sera étudiée. Enfin, les données récoltées dans le cadre de cette étude contribueront à une meilleure compréhension des mécanismes qui régulent les flux de matière à l'intérieur d'un écosystème peu étudié, démarche essentielle pour la préservation de notre environnement futur.

Cette étude permettra de fournir des éléments de réflexion :

- Aux collectivités locales cherchant des solutions durables et satisfaisantes pour l'élimination de déchets en production croissante, tout en voulant préserver l'environnement
- Aux citoyens soucieux de préserver leur droit d'accès à des zones de loisirs et récréatives naturelles de bonne qualité
- Aux écologues qui veulent développer leur connaissance du fonctionnement des systèmes naturels et des impacts des activités humaines sur celui-ci.

Elle s'articule en deux parties. La première partie concerne un apport de compost en garrigue sans destruction de la végétation, avec un double objectif de restauration de l'écosystème et de reconstitution de sol. La deuxième partie consiste à étudier les potentialités de reboisement offertes par un apport de compost dans une plantation.

Ce travail de thèse est présenté sous forme d'articles indépendants et complémentaires, chaque article constituant un chapitre au sein de chaque partie.

L'effet du compost sur l'écosystème de garrigue sera abordée successivement au travers de l'étude des modifications :

- du sol,
- de la nutrition des plantes,
- de leur recouvrement et de leur biomasse,
- des flux d'azote et de phosphore dans le système sol-plantes,
- ainsi que de la décomposition de la litière de l'espèce majoritaire (*Q. coccifera*).

Par ailleurs, l'effet du compost sur le reboisement en garrigue fait intervenir plusieurs espèces d'arbres étudiées dans deux articles différents.

Chapitre 2

ÉTAT DE L'ART

1. Amendements organiques en forêt et en milieu naturel

1.1 Boues d'épuration en forêt

L'utilisation des boues de stations d'épuration en forêt s'est considérablement développée dans les pays tels que les USA, le Canada, la Suède et la Nouvelle Zélande, où la forêt est considérée comme un système de culture à rôle productif. Le but recherché est alors l'augmentation du rendement. Pour les expérimentations conduites en Grande Bretagne, [Mc Kay & Moffat \(2001\)](#) notent qu'en général, toutes les formes d'application de boues d'épuration ont effectivement augmenté la croissance des arbres. En outre, l'enrichissement en MO entraîne des rendements plus élevés qu'un fertilisant minéral à taux d'éléments fertilisants égal ([Chen et al. 1996](#)).

En France, l'expertise scientifique en matière d'épandage des boues en forêt est extrêmement limitée ([INRA 2001](#)). En France, la filière bois n'est pas vraiment demandeuse pour l'utilisation de déchets en forêt. La demande vient le plus souvent de l'extérieur et en particulier des collectivités, ou de la filière de traitement des déchets ([Marien 2000](#)). Mais plusieurs perspectives de valorisation sont envisageables : boisement des friches agricoles, création de boisements spécialisés intensifs à fort pouvoir de filtre et haute valeur ajoutée, et enfin, comme dans le cas de notre expérimentation, la restauration de terrains dégradés et la plantation d'espèces moins sensibles aux incendies ([Marien 2000](#)).

Pour pallier ce manque de données, un référentiel national sur l'épandage des boues en ligniculture a été mis en place dans le cadre de l'AIP-AGREDE (Action Incitative Programmée – Agriculture et Epandage des Déchets) impliquant l'INRA, l'ADEME et d'autres grands organismes de recherche.

1.2 Région méditerranéenne

En région méditerranéenne, les sols sont généralement pauvres en matière organique, et les amendements riches en matière organique stable, tels les composts, sont bien adaptés. Quelques études espagnoles portent sur l'effet d'amendements organiques (compost d'ordures ménagères, compost de boues d'épuration, boues brutes) en milieu naturel ([Navas et al. 1999](#) ; [Guerrero et al. 2001](#) ; [Martinez et al. 2003^a](#) ; [Moreno-Peñaranda et al. 2004](#)). Cependant, ces travaux se focalisent principalement sur le compartiment sol, certains

paramètres concernant la végétation naturelle n'étant pas toujours pris en compte (recouvrement, biomasse, nutrition, composition floristique). De plus, ces études sont réalisées principalement sur des espèces herbacées, dans des zones où le couvert végétal est très faible (juste après incendie, sur des terrains très dégradés, ou bien dans le cas de restauration de carrières). Les auteurs ont généralement mis en évidence un accroissement du recouvrement, de la production de biomasse et du contenu nutritif de la végétation après amendement.

En résumé, peu de références sur les réactions des espèces méditerranéennes de garrigue sont disponibles, et peu d'études abordent les effets engendrés d'un point de vue global permettant d'approcher les modifications du fonctionnement de l'écosystème.

En ce qui concerne les expérimentations dans le sud de la France, quelques essais de reboisement ont été conduits par le CEMAGREF et la Société du Canal de Provence dans les années 80 (Ville de Marseille 1988 ; Ripert 2000^a ; Ripert 2000^b). Pour tous les essais, l'effet de l'apport organique (compost d'ordures ménagères, boues déshydratées ou brutes, doses élevées, apport en surface) est favorable sur la croissance. Cet effet semble principalement dû à l'amélioration du bilan hydrique constaté au niveau du sol suite à l'amendement.

2. Effets des composts de boues sur le sol

2.1 Propriétés physiques

Le mélange du compost avec le sol, matériau de plus faible densité et riche en matière organique plus ou moins fragmentée, améliore la structure du sol (Bouanani 2001), augmente sa porosité totale et diminue sa densité apparente (Pagliali *et al.* 1981). En effet, il se forme de nouveaux agrégats stables par adhésion des particules du sol aux molécules organiques (notamment les acides humiques) (Pagliali & Antisari 1993 ; Zahrer & Lawrence 2001). Ainsi, le développement des racines des plantes est amélioré (aération et espace), la vie du sol optimisée (microorganismes, macro et microfaune du sol) et l'érosion par ruissellement est limitée par une meilleure pénétration de l'eau dans le sol. D'autre part, la capacité de rétention en eau du sol augmente, ce qui présente un intérêt certain en région méditerranéenne où le stress hydrique est le premier facteur limitant la croissance des végétaux.

2.2 Matière organique

Les composts à base de boues contiennent de 20 à 30 % d'humus (MS) (Ministère de l'Environnement 1983), dont les acides humiques se différencient de ceux du sol par leur stabilité, leur taille et leur acidité plus faibles, et leur teneur moins importante en composés phénoliques (Garcia *et al.* 1991). L'apport de compost augmente la CEC proportionnellement à la dose d'apport (Epstein *et al.* 1976). Une augmentation de la CEC du sol augmente le pool de nutriments potentiellement disponibles pour les végétaux (cations) et diminue le lessivage des ETM, qui sont préférentiellement fixés sur le complexe adsorbant plutôt que transférés vers la solution du sol.

2.3 Vie du sol

L'apport de compost a un effet positif sur l'activité des microorganismes du sol à long terme. Il augmente généralement leur respiration spécifique, leur biomasse carbonée et leurs activités enzymatiques (Zahrer & Lawrence 2001).

2.4 Eléments fertilisants

Dans le cas d'un épandage de boues compostées, la minéralisation de l'N apporté est très lente et proche de celle d'un sol sans amendement (Epstein *et al.* 1978). Moins de 20 % de l'azote apporté par un compost de boues est minéralisé, et donc disponible, la première année après l'épandage. Souvent, les valeurs trouvées sont même inférieures à 20 % (de l'ordre de 10 %). Les boues sont des composés riches en sels. On trouve dans les composts de boues des teneurs moyennes en CaO de 4 % de MS, avec une forte variabilité (ajout de chaux ou pas). En ce qui concerne la potasse K₂O, les quantités moyennes dans les composts de boues sont de l'ordre de 0,5 % de MS (Ministère de l'Environnement 1983). Cette valeur faible est due à la grande solubilité de la potasse, qui a tendance à rester dans les effluents traités lors de l'épuration et ne s'accumule donc pas dans les boues.

2.5 Eléments trace métalliques

Les boues de stations d'épuration contiennent des proportions variables d'éléments trace métalliques en fonction de la taille des stations d'épuration dont elles sont issues (nombre d'équivalents habitants) et de la nature plus ou moins industrielle des activités urbaines de la zone desservie. Préalablement à l'amendement, les sols peuvent contenir des ETM en plus ou moins grande quantité selon d'une part la nature de la roche mère, d'autre part l'intensité des

pollutions anthropiques diffuses auxquelles ils sont soumis, tout cela en relation avec leur capacité intrinsèque à retenir les éléments métalliques (teneur en argiles, CEC).

La mobilité d'un élément donné est en relation avec sa spéciation, c'est à dire sa distribution entre les différentes formes ou espèces chimiques sous lesquelles il peut exister dans le milieu (Bourg 1995). Les réactions d'hydrolyse et de complexation dissoute augmentent la solubilité des métaux et donc leur mobilité, alors que les réactions de précipitation, d'adsorption et d'absorption par les êtres vivants (bioaccumulation) diminuent leur solubilité et leur transport (Bourg 1995). Certains paramètres du système biogéochimique sol déplacent l'équilibre de ces réactions chimiques et contrôlent donc le comportement des ETM dans le sol. Le facteur principal est le pH du sol, dont la diminution provoque la mobilisation des métaux par échange protonique, la solubilisation de sels insolubles (précipités), et la destruction de la phase de rétention des cations métalliques (diminution de la CEC) (ADEME 1995).

L'apport de compost change la distribution des ETM dans le sol. Cela est mis en évidence dans de nombreuses expérimentations au moyen de procédés d'extraction chimique séquentielle. En effet, les fractions d'ETM liées aux précipités inorganiques (pour le Pb, le Cr et le Zn surtout) et à la MO (Cu, Ni, Zn) augmentent au détriment de la fraction la moins disponible, dite résiduelle (Sims & Kline 1991 ; Pitchel & Anderson 1997 ; ADEME 2000). Cependant, la fraction d'ETM retrouvée dans la fraction soluble reste faible (ADEME 2000).

3. Effets des amendements organiques sur le végétal

Les effets d'un amendement organique sur les plantes sont bien documentés, dans des contextes de sylviculture, d'horticulture ou agricoles.

L'application de compost a un effet bénéfique sur la nutrition, la croissance et le rendement des végétaux en général, pour des doses d'apport raisonnables et des composts peu contaminés par des substances toxiques telles les métaux traces (Gouin & Walker 1977 ; Brockway 1983 ; Planquart *et al.* 1999). L'effet fertilisant est souvent proportionnel à la dose d'apport (Dutch & Wolstenholme 1994 ; Luo & Christie 1995) et semble s'estomper au bout de quelques années (Pillard-Landeau 2001).

Les effets toxiques des éléments traces métalliques (ETM) sur les végétaux ont été largement étudiés. Certains ETM sont des oligoéléments pour les plantes, c'est à dire qu'ils sont indispensables en faible quantité à la réalisation de certains processus vitaux (inférieure à 100 mg.kg⁻¹ MS). Ce sont le bore (B), le chlore (Cl), le calcium (Ca), le cuivre (Cu), le fer (Fe), le

manganèse (Mn), le molybdène (Mo), le sodium (Na), le nickel (Ni) et le zinc (Zn). D'autres sont néfastes pour les processus biologiques (le plomb Pb, le mercure Hg et le cadmium Cd) (ADEME 1995). Peu d'informations sont disponibles au sujet des seuils de toxicité de ces éléments pour le végétal. La phytotoxicité métallique se traduit en général par une diminution de la production de biomasse. Cette baisse est liée à des déficiences en macronutriments dans le sol causées premièrement par le déplacement des cations basiques par les cations métalliques, deuxièmement par le retard dans la décomposition et la minéralisation de la litière (effets toxiques sur les microorganismes) et troisièmement par la diminution de l'absorption des nutriments liée aux perturbations du fonctionnement des racines fines et des mycorhizes (Mälkönen *et al.* 1999). A l'intérieur des tissus, les métaux provoquent des phénomènes oxydatifs (formation de radicaux libres induisant des mutations des acides nucléiques, des dysfonctionnements enzymatiques et la peroxydation des lipides) et génotoxiques (carcinogénèse) (Briat & Lebrun 1999).

Chapitre 3

APPROCHE EXPERIMENTALE

1. Cadre conceptuel : succession végétale, perturbations et résilience

L'observation des communautés végétales dans l'espace et le temps a conduit à l'émergence du concept écologique de succession végétale (Clements 1916, *in* Lepart & Escarré, 1983), dont l'essence est de donner un patron commun de mise en place et d'évolution de la végétation. Le concept théorique de succession définit un mécanisme linéaire d'évolution. Cette linéarité est rompue par l'occurrence de perturbations (Cooper 1926, *in* White & Jentsch 2001) de régimes variés, qui introduisent des ramifications et ouvrent le champ de la dynamique de végétation à la diversification. La résilience (Westman 1978, *in* Westman 1986) apparaît comme la notion régissant la capacité de retour à l'évolution linéaire à partir des ramifications possibles empruntées après perturbation.

L'utilisation de ces trois notions écologiques nécessite de fixer un cadre d'étude et de définition spatio-temporel précis, au vu de la diversité des approches possibles, fonction de l'échelle choisie.

1.1 Succession végétale

La succession végétale est définie comme le processus de colonisation d'un biotope par un ensemble de végétaux, occasionnant des changements dans le temps de la richesse et de la composition spécifique et fonctionnelle pour la communauté considérée, après une perturbation ou une modification liée à la dynamique interne du système (Quézel & Médail 2003).

L'étude de la succession végétale implique deux approches possibles (Lepart & Escarré 1983) :

- Approche directe ou diachronique, qui consiste à observer dans le temps les modifications de la végétation d'une station. Cette approche est difficilement réalisable à l'échelle d'une vie humaine.
- Approche indirecte ou synchronique, où les communautés sont observées au même moment dans des lieux différents constituant un espace plus ou moins homogène et sont ordonnées en séquences ou séries de végétation. Cette approche implique de

choisir des stations à climat, substrat, environnement et régime de perturbation homogènes, ce qui est rarement le cas.

Dans le cas des successions secondaires, la plupart des espèces qui colonisent en premier sont déjà présentes sur le site immédiatement après perturbation (Chapin *et al.* 2002). Par ailleurs, l'intensité ou la durée de la perturbation permet de définir des successions régénératives au sein des successions secondaires. Dans ce cas, les perturbations sont modérées avec persistance *in situ* d'une banque de graines et rejet de souche des ligneux (Quézel & Médail 2003).

La végétation de garrigue de l'Arbois s'inscrit dans une dynamique de succession régénérative après incendie, que l'on peut aussi qualifier d'autosuccession du fait de la conservation de la composition floristique initiale de la communauté végétale après incendie.

Différents modèles ont été développés pour expliquer les processus de succession, dont les modèles de Connell et Slatyer, ainsi que celui de Grime que nous utiliserons dans cette étude.

Connell et Slatyer (1977, *in* Lepart & Escarré 1983 ; *in* Quézel & Médail 2003) ont orienté leur analyse vers le rôle de la compétition interspécifique dans les changements de la composition spécifique. Ils ont défini trois modèles :

- Modèle de facilitation : les espèces appartenant à ce modèle jouent un rôle favorable dans l'implantation et le développement des végétaux des phases successives ultérieures.
- Modèle de tolérance : les espèces appartenant à ce modèle colonisent le biotope indépendamment de l'influence sur le milieu des espèces pionnières.
- Modèle d'inhibition : ces espèces empêchent ou limitent l'installation d'autres espèces.

Grime (1979, *in* Hodgson *et al.* 1999 ; *in* White & Jentsch 2001) répartit la flore selon trois pôles, en fonction de deux contraintes environnementales principales qui affectent les performances des plantes :

- La disponibilité des ressources, qui définit un gradient de stress.
- La perturbation qui entraîne une destruction de biomasse.

Les rudérales colonisent les habitats caractérisés par des perturbations sévères et fréquentes. Les stress-tolérantes colonisent les habitats drastiques et pauvres en nutriments où les ressources sont imprévisibles et brèves, donc limitées. Enfin, les compétitrices colonisent les environnements peu perturbés et à faible stress, où la disponibilité des ressources est maximale.

1.2 Perturbations

Les perturbations sont des événements ubiquitaires, inévitables, qui affectent tous les niveaux d'organisation biologiques. Actuellement, les écologistes ne les considèrent plus comme des forces extraordinaires et globalement destructrices. En effet, elles peuvent avoir une variété de causes et d'effets quantitatifs et qualitatifs. De plus, elles constituent des forces d'évolution qui jouent un rôle majeur dans le maintien de la diversité biologique (White & Jentsch 2001).

Une perturbation peut être définie comme un événement causant une déviation de la dynamique d'un écosystème. Cette définition relative repose souvent sur le postulat de base de l'existence d'une dynamique « normale », difficile à valider. Elle s'appuie sur l'analyse statistique du passé et pose le problème de l'échelle spatiale et temporelle la plus appropriée pour s'affranchir des variations.

En conséquence, une définition absolue a été donnée, qui s'appuie sur l'observation des changements réels de variables physiques et mesurables au moment de la perturbation. Par exemple, Grime a défini la perturbation comme une réduction partielle ou complète de biomasse (White & Jentsch 2001).

Dans le cas de cette étude, la perturbation sera considérée comme la force appliquée à la végétation, et non comme la réponse de la végétation (Dell *et al.* 1986). De plus, nous retenons la définition de Grime, qui s'applique à l'étude de la végétation, et pour laquelle la perturbation constitue une perte de biomasse. Ainsi, l'écrasement s'étant produit au moment de l'épandage constitue une perturbation car il induit une diminution du recouvrement global de la végétation. Toutes les espèces sont soumises à la même intensité de perturbation, mais les réponses peuvent être différentes.

Par ailleurs, une des caractéristiques importantes des perturbations est leur infréquence, relativement au temps qui sépare deux événements, et leur brièveté, en relation avec la durée de vie des espèces qu'elles affectent. Ce point est primordial pour séparer les perturbations des stress, processus qui affectent la structure de l'écosystème sans une influence directe et abrupte (White & Jentsch 2001).

En région méditerranéenne, la sécheresse joue un rôle majeur dans la dynamique et la structuration de la végétation. Elle constitue un stress saisonnier, plutôt qu'une perturbation car elle intervient à intervalles réguliers (Quézel & Médail 2003). Il est donc très important de définir la perturbation en tenant compte du contexte de variation environnementale habituel d'un écosystème (Chapin *et al.* 2002).

1.3 Résilience

La résilience est définie comme la vitesse, la façon et le degré de restauration d'un écosystème pour une propriété donnée après une perturbation (Westman 1986). Cependant, selon Gunderson *et al.* (2002), il existe une deuxième manière de définir la résilience, en considérant qu'elle constitue la quantité de perturbation qu'un système peut absorber avant qu'il ne change d'état (résilience écologique). Dans cette étude, nous considérerons que cette deuxième approche correspond plutôt à l'inertie d'un écosystème, définie par Westman (1986). Inertie et résilience nécessitent de définir précisément le patron des fluctuations naturelles des propriétés d'un écosystème en l'absence de perturbations majeures (concept de stabilité, Westman 1986).

Comme l'objectif de cette étude est centré autour des potentialités de développement de la strate arborée après apport de compost, le terme de résilience correspond ici au temps nécessaire à la réinstallation des espèces arborées naturelles ayant disparu en raison de la récurrence des feux. En ce qui concerne la « cicatrisation » de la végétation après la perturbation « écrasement », on utilisera plutôt le terme de régénération, qui fait référence à une échelle de temps beaucoup plus restreinte.

2. Définition des paramètres à étudier

Un écosystème est un système ouvert en équilibre dynamique avec les conditions environnementales auxquelles il est soumis. Au sein d'un écosystème existe une hiérarchie de

niveaux fonctionnels (producteurs = végétaux, herbivores, prédateurs, décomposeurs) entre lesquels l'énergie et la matière circulent (Schulze 1995). Dans le cadre de cette étude, nous focaliserons nos investigations sur le système sol / plantes. Afin de déterminer les modifications induites par l'amendement sur le recyclage des éléments, les trois compartiments fonctionnels de ce système (sol, végétation, litière) seront étudiés.

1.1 Le sol

L'amendement est susceptible de modifier les 3 types de facteurs du sol influençant la productivité végétale, définis par Martin-Prével *et al.* (1984) :

- Facteurs physiques (énergétiques et mécaniques) : texture et structure, lumière arrivant au sol, température.
 - Facteurs chimiques (approvisionnement ou échanges de matière) : humidité du sol, nutriments, éléments potentiellement toxiques, corps organiques.
 - Facteurs biotiques : apport de microorganismes exogènes.
- Dans cette étude seront principalement prises en compte les modifications des facteurs chimiques induites par l'apport de compost. Ces modifications seront abordées dans le cas de deux types d'épandage différents : en surface, et en mélange au sol.
 - Dans le cas de l'épandage en surface, l'étude du sol sera menée en parallèle sur les fractions litière, humus et horizon minéral.

1.2 La végétation

En réponse à la modification du compartiment sol après amendement, la nutrition, la productivité et les relations interspécifiques au sein de la végétation sont susceptibles de changer. Ces paramètres seront étudiés en tentant de répondre aux questions suivantes, qui abordent les conséquences de l'amendement sur la dynamique de la végétation et sa résilience après perturbation :

Quelles est la réactivité des espèces dominantes de la garrigue à une augmentation du niveau de ressources, et notamment de celles pouvant ralentir la dynamique successione et la résilience du système à long terme ? Quelles vont être les espèces qui vont bénéficier le plus des effets de l'amendement et quels changements pour l'écosystème peuvent découler de l'amélioration des paramètres abiotiques ?

Pour répondre à ces questions, une expérimentation d'apport de compost en garrigue, sans destruction de la végétation est réalisée. Les espèces suivies sont principalement *Quercus coccifera*, *Brachypodium retusum*, *Cistus albidus* et *Ulex parviflorus*, et dans une moindre mesure *Cistus salviaefolius* et *Rosmarinus officinalis*.

L'apport de compost en milieu dégradé peut-il créer des conditions favorables à l'installation des espèces arborées naturelles en garrigue ?

Cette question fait l'objet d'une deuxième expérimentation avec apport de compost dans une plantation de *Quercus ilex*, *Quercus pubescens*, *Pinus halepensis* et *Pinus pinea*.

- La productivité des principales espèces de la garrigue sera étudiée à travers la mesure du recouvrement et l'estimation des biomasses dans l'expérimentation en garrigue, à travers la croissance en hauteur et en épaisseur (diamètre caulinare) dans l'expérimentation plantation.

Biomasse et recouvrement des plantes sont associés à des processus importants de l'écosystème, tels que les flux d'énergie et l'équilibre hydrique, l'érosion, la structure de l'habitat, mais surtout aux cycles des nutriments (Diaz-Delgado *et al.* 2002).

- L'étude de la nutrition végétale viendra compléter celle de la productivité pour estimer les quantités d'éléments du compost qui transitent par le compartiment végétation.
- Les modifications des relations entre espèces végétales en garrigue seront abordées à travers le suivi de l'évolution relative des recouvrements et biomasses des espèces majeures après amendement, ainsi qu'au travers des réponses de la végétation à l'amendement vis-à-vis de leur nutrition.

L'apport de compost est susceptible de modifier les proportions relatives d'espèces appartenant à des groupes fonctionnels différents, dont la réponse à une disponibilité accrue

des nutriments sera variable. En effet, les relations de compétition interspécifique sont déterminées par les caractéristiques morphologiques propres à chaque espèce, qui déterminent leur aptitude à utiliser les ressources du milieu (Wardle *et al.* 1998). En ce qui concerne l'utilisation des nutriments, des espèces d'une même communauté peuvent utiliser des sources différentes, comme les plantes avec des bactéries fixatrices d'azote, les plantes capables d'utiliser l'azote organique, ou les plantes mycorhizées qui ont un accès privilégié au P lié à la matière organique (Hooper 1998).

- L'évaluation des réactions de la végétation à l'amendement sera donc effectuée par espèce, mais également en organisant les réponses de ces différentes espèces par groupes fonctionnels.

Les groupes fonctionnels sont ici définis comme des groupes d'espèces ayant des traits de vie communs induisant une réponse similaire à un facteur environnemental donné. Les traits de vie les plus pertinents sont alors ceux qui optimisent la perception de cette association entre végétation et variation environnementale (Pillar 1999).

1.3 Les litières

La dynamique des litières constitue un aspect important des cycles des nutriments et des transformations d'énergie dans les systèmes naturels (Kavvadias *et al.* 1999) car elle conditionne la restitution au sol des éléments absorbés par la végétation. L'apport de compost est susceptible de modifier quantitativement et qualitativement le compartiment litière. En effet, l'amélioration des paramètres édaphiques peut augmenter la production de biomasse et donc la fraction de cette biomasse restituée au sol. De plus, l'amélioration possible de la nutrition azotée des végétaux se développant sur sol amendé pourrait accélérer la minéralisation de la litière. En effet, plus la litière est riche en azote, plus la vitesse de décomposition est importante (Aerts 1997 ; Wardle *et al.* 1998). D'autre part, le compost enrichit le sol en humus et litière exogènes de nature différente par rapport à la matière organique produite par la végétation en place. Le compost contient des décomposeurs particuliers qui peuvent entrer en compétition avec les décomposeurs endogènes du sol et provoquer des modifications importantes dans le recyclage de la matière au sein de l'écosystème initial.

- Ce compartiment sera pris en compte dans le cas d'un épandage de compost en surface.
- L'effet du compost sur la colonisation des feuilles de la principale espèce ligneuse de la garrigue, *Quercus coccifera*, par les microorganismes décomposeurs sera également étudié.

En effet, la biomasse microbienne est souvent considérée comme un indicateur précoce des changements qui peuvent se produire après un changement dans la fertilité du sol (Wardle *et al.* 1999).

3. Présentation du site expérimental

2.1 Généralités

2.1.1 Localisation

Le plateau de l'Arbois se trouve dans le département des Bouches-du-Rhône, au sud ouest d'Aix en Provence (Figure 1). Il est entouré des zones industrielles de Fos / Etang de Berre à l'ouest et d'Aix-Les Milles au nord est. Il culmine à 260 m d'altitude. Le site expérimental s'étend sur environ 4 hectares (Photo 1).

2.1.2 Climat

Le climat de cette zone est typiquement méditerranéen, avec une sécheresse estivale prononcée (mois les plus secs : juin / juillet / août) et des pluies irrégulièrement réparties sur l'année (66% en moyenne des chutes de pluie sont réparties sur 4 mois : septembre, octobre, novembre et avril). La température annuelle moyenne est de 13°C et les précipitations annuelles moyennes de 650 mm (CEMAGREF 1983). D'autre part, le plateau est fortement exposé au vent, notamment au mistral.

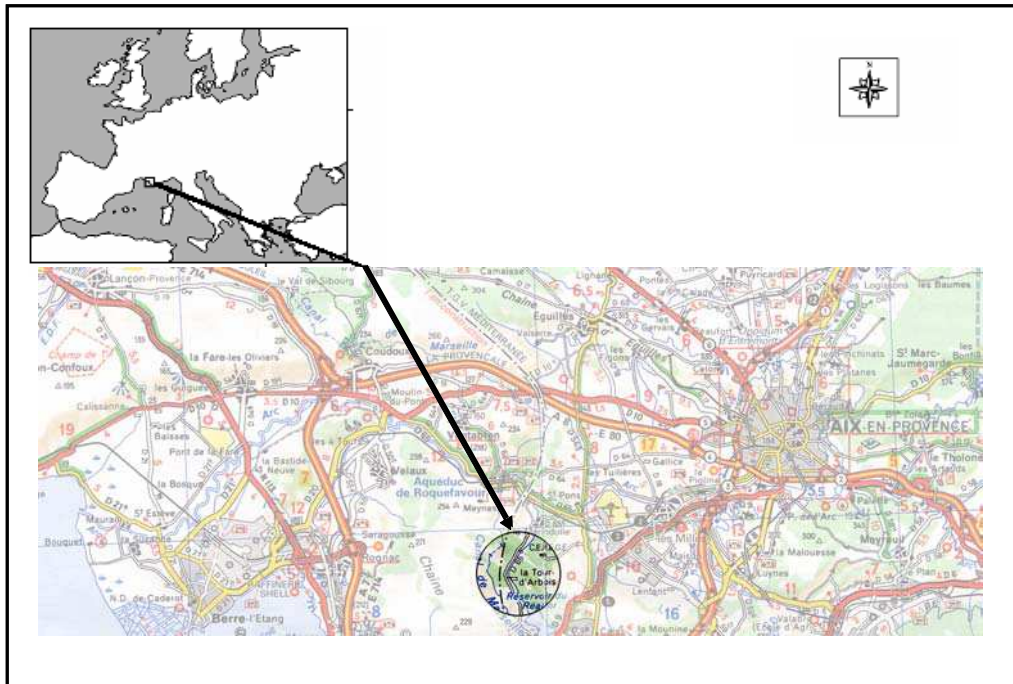


FIGURE 1

LOCALISATION GEOGRAPHIQUE DU PLATEAU DE L'ARBOIS
(CARTE MICHELIN, BOUCHES DU RHONE - VAR, T340)

PHOTOGRAPHIE 1

VUE GENERALE DU SITE EXPERIMENTAL



2.1.3 Végétation

L'Arbois a fréquemment été incendié par le passé, le dernier incendie s'étant produit en 1995. Le sol, squelettique et peu profond, est de type rendzine sur roche mère calcaire dure (thanétien). Le site est colonisé par une garrigue haute à chêne kermès (*Quercus coccifera* L.), brachypode rameux (*Brachypodium retusum* Pers.), cistes (*Cistus albidus* L. et *salviaefolius* L.) et ajonc de Provence (*Ulex parviflorus* Pour.), ces espèces appartenant à la série du chêne vert (*Quercus ilex* L.) et du chêne pubescent (*Quercus pubescens* Miller).

2.1.4 Contamination en éléments trace métalliques

Le sol du site est relativement contaminé en Ni, Cr et Pb par rapport aux teneurs limites imposées avant épandage par l'arrêté du 8 janvier 1998. L'origine de cette contamination est probablement atmosphérique, du fait de la répartition homogène de ces polluants dans le sol d'une part, et de la proximité de la zone industrielle de l'étang de Berre d'autre part. En effet, un déplacement de masses d'air polluées (SO₂) entre Berre et Les Milles a été récemment mis en évidence par une étude d'[AIRMARAIX \(1999\)](#). [Hernandez et al. \(2003\)](#) rapportent que les zones forestières françaises a priori non polluées recevant une pollution acide significative sont souvent contaminées en Cr et Pb, notamment dans le cas de sols calcaires qui « piègent » les éléments traces.

2.2 *Expérimentations*

Deux expérimentations complémentaires avec utilisation de compost ont été menées en parallèle sur le site :

- une plantation avec apport de compost en mélange au sol au pied de chaque plant
- un épandage en surface dans la garrigue, sans destruction de la végétation en place

Les deux expérimentations sont géographiquement proches et sur des terrains similaires vis-à-vis des conditions pédoclimatiques.

2.3 *Plantation*

Elle s'étend sur trois hectares de plateau et environ 2500 m² en pente.

2.3.1 Préparation du site

La préparation du site a consisté à débroussailler les futures lignes de plantation espacées de trois mètres. Ensuite, des trous de un mètre carré ont été creusés à quatre mètres d'intervalle sur ces lignes, chaque trou correspondant à l'emplacement d'un plant.

2.3.2 Epannage

Un apport unique de compost a été effectué en mai 2001. Le compost a été mélangé avec le sol dans chaque trou à la pelleteuse. Le sol des trous du block D0 fut mélangé mécaniquement également.

En considérant une densité de plantation de 400 plants par hectare et l'apport de compost étant effectué au pied de chaque plant, il a été globalement épanché 8 (D20) et 16 (D40) tonnes de compost brut par hectare sur les deux blocs amendés.

2.3.3 Plantation

Sept mois après amendement (décembre 2001), quatre espèces d'arbres ont été plantées : *Quercus ilex* L., *Pinus halepensis* Miller et *Pinus pinea* L. sur le plateau et *Quercus pubescens* L. dans la pente. Au moment de la plantation, les plants avaient une saison de croissance.

Au pied de chaque plant, une protection cellulosique de 1600 cm² a été posée sur le sol pour limiter les pertes en eau du sol et le développement de la végétation adventice.

2.3.4 Plan expérimental

Le plan expérimental fait intervenir deux facteurs : la dose de compost et l'espèce plantée.

Sur le plateau, trois blocs de un hectare correspondent chacun à une dose de compost : D0 sans compost, D20 avec 20 kg de compost brut par plant et D40 avec 40 kg de compost brut par plant (soit 0, 200 et 400 t de compost brut à l'hectare localement au pied des plants).

A l'intérieur de chaque bloc (D0, D20, D40), neuf sous-parcelles de 40m x 15m, soit trois rangs de dix plants, ont été disposées au hasard, chacune correspondant à une espèce plantée (trois par espèce au minimum) (Figure 2).

Dans la pente, trois blocs de 200 m², soit cinq lignes de dix plants chacune, correspondent aux trois doses de compost précitées : D0, D20 et D40. Les blocs D0 et D40 sont situés dans le même thalweg, le bloc D0 dominant le bloc D40. Le bloc D20 se trouve dans un thalweg un peu plus à l'ouest du premier.

2.3.5 Suivi effectué (Tableaux 1 et 2)

TABLEAU 1

CALENDRIER DES PRELEVEMENTS DANS LA PLANTATION : MELANGE SOL-COMPOST

| | 2001 | 2002 | 2003 |
|-----------|--|----------------------------|-----------------------------|
| janvier | | | |
| février | | | |
| mars | | | |
| avril | | | |
| mai | Etat initial avant épandage Epannage | | |
| juin | | | |
| juillet | | | |
| août | | | |
| septembre | | | |
| octobre | | | |
| novembre | | | Etat 2 ans après plantation |
| décembre | Etat 7 mois après épandage Plantation | Etat 1 an après plantation | |

Analyses agronomiques + contamination en métaux traces

TABLEAU 2

CALENDRIER DES PRELEVEMENTS DANS LA PLANTATION : VEGETAUX

(4 ESPÈCES : *Pinus halepensis*, *Pinus pinea*, *Quercus ilex*, *Quercus pubescens*)

| | 2001 | 2002 | 2003 |
|-----------|---|----------------------------|-----------------------------|
| janvier | | | |
| février | | | |
| mars | | | |
| avril | | | |
| mai | | | |
| juin | | | |
| juillet | | | |
| août | | | |
| septembre | | | |
| octobre | | | |
| novembre | | | Etat 2 ans après plantation |
| décembre | Etat initial au moment de la plantation | Etat 1 an après plantation | |

Survie (1 an et 2 ans après plantation) + Biométrie
Analyses nutritionnelles (N, P, cations) + contamination en métaux traces

2.4 Garrigue

La zone expérimentale s'étend sur 6000 m², soit douze parcelles de 500 m² chacune.

2.4.1 Epannage

L'épannage a été effectué en surface, sans débroussaillage préalable, en janvier 2002. Deux doses de compost ont été choisies : 50 tonnes et 100 tonnes par hectare.

2.4.2 Dispositif expérimental

Le dispositif expérimental constitue un bloc de répétitions : 4 parcelles choisies au hasard parmi les douze correspondent à une des deux doses de compost ou à un témoin sans apport (Figure 3).

2.4.3 Suivi effectué (Tableaux 3, 4 et 5)

TABLEAU 3
CALENDRIER DES PRELEVEMENTS EN GARRIGUE : SOL ET COMPOST
(3 FRACTIONS : SOL, HUMUS, LITIERE)

| | 2001 | 2002 | 2003 | 2004 |
|-----------|------|-----------------------------|--------------------------|---------------------------|
| janvier | | Epannage en surface | | |
| février | | | | |
| mars | | Etat après épannage | Etat 1 an après épannage | Etat 2 ans après épannage |
| avril | | | | |
| mai | | | | |
| juin | | | | |
| juillet | | | | |
| août | | | | |
| septembre | | | | |
| octobre | | | | |
| novembre | | Etat initial avant épannage | | |
| décembre | | | | |

| | |
|--|--|
| | Analyses agronomiques + contamination en métaux traces |
| | Analyses agronomiques |

FIGURE 3

ZONE DE GARRIGUE : DISPOSITIF EXPERIMENTAL

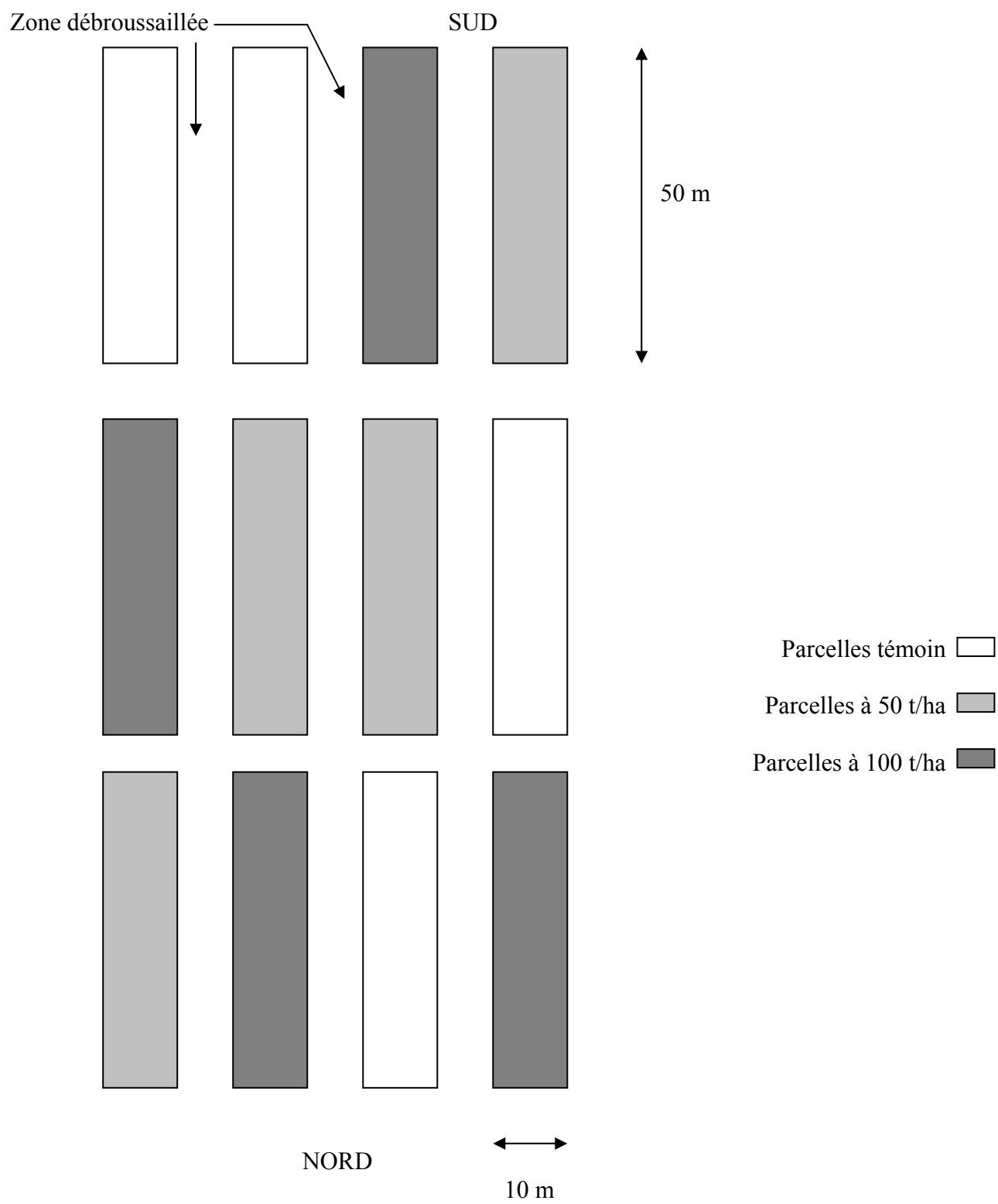


TABLEAU 4

CALENDRIER DES PRELEVEMENTS EN GARRIGUE : VEGETAUX

(4 ESPECES : *Quercus coccifera*, *Cistus albidus*, *Ulex parviflorus*, *Brachypodium retusum*)

| | 2002 | 2003 | 2004 |
|-----------|-----------------------|--------------------------|---------------------------|
| janvier | Epandage en surface | | |
| février | | | |
| mars | Etat avant croissance | Etat 1 an après épandage | Etat 2 ans après épandage |
| avril | | | + Biomasses |
| mai | | | |
| juin | | | |
| juillet | | | |
| août | | | |
| septembre | | | |
| octobre | | | |
| novembre | | | |
| décembre | | | |

| | |
|--|---|
| | Analyses nutritionnelles + contamination en métaux traces Recouvrement |
| | Analyses nutritionnelles Recouvrement |
| | Analyses nutritionnelles (N, P, cations) |

TABLEAU 5

CALENDRIER DES PRELEVEMENTS EN GARRIGUE : BIOMASSES BACTERIENNE ET FONGIQUE

DE LA LITIERE LIBRE DE *Quercus coccifera*

| | 2002 | 2003 |
|-----------|---------------------|--------------------------|
| janvier | Epandage en surface | |
| février | | |
| mars | Etat après épandage | Etat 1 an après épandage |
| avril | | |
| mai | | |
| juin | | |
| juillet | | |
| août | | |
| septembre | | |
| octobre | | |
| novembre | | |
| décembre | | |

| | |
|--|---------------------------------|
| | Biomasse fongique |
| | Biomasse fongique + bactérienne |

4. Présentation du compost

3.1 Fabrication

Le compost utilisé est produit par la société Biotechna, sur le site de compostage d'Ensues La Redonne (Bouches du Rhône). Il est obtenu par mélange de trois volumes égaux de boues (biologiques, aérobies), de déchets verts broyés et d'écorces de pin (favorisant l'aération du mélange à composter et apportant les molécules de cellulose et de lignine à la base de la formation des composés humiques), plus un demi-volume de broyat de palettes alimentaires.

3.2 Boues utilisées

Les boues sont d'origine locale et proviennent entre autres des stations d'épuration de la Palun et de Vitrolles. Elles ont subi un traitement tertiaire de dénitrification et de déphosphatation. Le traitement tertiaire de déphosphatation (floculation avec du chlorure de Fe) influence grandement la répartition du P dans les différentes fractions du sol et augmente la fraction immobilisée. Pour des boues sans traitement tertiaire, 50 % du P se trouve sous forme organique dans le compost, alors que pour un compost de boues issues d'une épuration avec déphosphatation, la plus grande partie du P est associée au Fe et seulement 2 à 5 % est sous forme organique.

3.3 Caractéristiques agronomiques (cf. Table 1 des articles)

Le compost utilisé est relativement mûre, puisque sa phase de maturation dure plus de cinq mois. Il est riche en calcium et son pH est basique, en relation avec la nature calcaire des roches de la région de traitement des eaux usées. Il se distingue par des teneurs en MO, N et P plus faibles que la moyenne des composts français (ADEME 1997), en relation avec la proportion réduite de boues entrant dans sa composition, ainsi que par un C/N peu élevé (13,4). En effet, plus le mélange de compostage contient de boues, plus le compost est enrichi en azote minéral et plus sa valeur finale en azote minéral est alors élevée (Sawhney *et al.* 1995). Cette teneur diminue également avec la maturité du compost (ADEME 2000).

3.4 Contamination

Le compost de Biotechna est faiblement contaminé par les ETM puisque les concentrations en métaux sont toutes inférieures à 13 % des taux limite autorisés dans les boues avant épandage agricole (arrêté du 8 janvier 1998). Les teneurs en polychlorobiphényles et en hydrocarbures

aromatiques polycycliques sont satisfaisantes car inférieures à 24 % des taux limite autorisés par l'arrêté du 8 janvier 1998.

3.5 Normalisation

Au moment de la mise en place de cette étude, le compost Biotechna bénéficiait d'une autorisation provisoire de vente car il était homologué. Actuellement, il est conforme à la norme NF U 44-095 sur les composts contenant des matières d'intérêt agronomique issues du traitement des eaux.

PREMIERE PARTIE
EPANDAGE DE COMPOST EN GARRIGUE

INTRODUCTION

La végétation de garrigue à *Quercus coccifera* qui s'installe sur les sites dégradés par le passage répété du feu est le signe d'une évolution régressive de la succession. Après chaque feu, les capacités de résilience diminuent et le laps de temps nécessaire à l'installation des espèces arborées augmente. La plupart des espèces de garrigue sont adaptées à un milieu de faible niveau de ressources. L'élévation du niveau de ressources liée à l'apport de compost dans un tel milieu va probablement entraîner des modifications dans les relations entre plantes en fonction de leur capacité spécifique de réaction, ainsi que dans le recyclage des éléments.

L'intérêt de cette étude en garrigue réside dans la considération des effets du compost à un niveau global de fonctionnement du système sol / plante, avec focalisation sur chaque compartiment fonctionnel et mise en relation. Tout d'abord, les modifications du compartiment sol (horizon minéral, humus, litière) suite à l'apport de compost seront suivies. Puis, l'attention se portera sur le compartiment végétation, au travers de l'étude de la nutrition et de la productivité des espèces majeures de la garrigue (*Quercus coccifera*, *Cistus albidus*, *Ulex parviflorus* et *Brachypodium retusum*) après amendement. Les conséquences de l'apport de compost sur le recyclage de l'azote et du phosphore au sein du système sol / plante seront abordées. Enfin, l'effet du compost sur la colonisation microbienne de la litière de *Quercus coccifera* sera étudié également.

Les résultats obtenus pour les différents paramètres étudiés au sein de chaque compartiment seront mis en relation dans la synthèse finale de la deuxième partie, qui mettra en exergue les points importants de la réponse du système garrigue à l'amendement, et qui discutera les limites de l'utilisation de compost de boues en milieu naturel. Notamment, le problème des contaminations possibles engendrées par l'apport de compost sera pris en compte à chaque stade d'étude. De plus, les effets du compost au niveau global de fonctionnement de l'écosystème seront abordés et la caractérisation des réponses des principales espèces de la garrigue sera effectuée.

Chapitre 1

L'EPANDAGE DE COMPOST PERMET-IL D'AMELIORER LES POTENTIALITES NUTRITIVES D'UN SOL DEGRADE DANS UN ECOSYSTEME MEDITERRANEEN ?

Le compost a été apporté en surface à trois doses : un témoin sans apport, une dose intermédiaire de cinquante tonnes de compost brut par hectare, et une dose maximale de cent tonnes de compost brut par hectare, sur un site colonisé par une végétation de garrigue à *Quercus coccifera* et *Brachypodium retusum*, sept ans après incendie.

En plus de la prise en considération de l'horizon A organo-minéral pour étudier les effets du compost sur le sol, nous avons choisi de suivre également les modifications de l'épisolum humifère (fraction humus <2mm et fraction litière >2mm, définies granulométriquement) après amendement. En effet, ces deux dernières fractions jouent un rôle primordial dans le recyclage des éléments au sein du système sol/plante et peu d'études les prennent en considération après amendement.

Nos objectifs étaient les suivants :

- Déterminer les effets de l'amendement sur la dynamique des paramètres de mesure de la fertilité au sein des fractions horizon A organo-minéral, humus et litière.
- Suivre les flux d'éléments issus du compost dans les trois fractions étudiées.
- Etudier la contamination éventuelle du sol par les métaux trace après amendement.

Remarque

Cette étude des effets du compost sur le sol incluait le suivi des teneurs en azote minéral des fractions horizon minéral et humus. Cependant, un événement imprévu a empêché la réalisation des analyses (décongélation accidentelle des échantillons).

Can sewage-sludge compost spreading improve the nutritional potential of a degraded soil in a Mediterranean ecosystem ?

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Article soumis le 21 octobre à The Science of the Total Environment

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Abstract

Mediterranean areas are prone to recurrent fires and violent precipitation events that favour erosion and induce soil impoverishment. Thus, Mediterranean degraded soils are often colonized by garrigues that are adapted to low resources availability and tree implantation is delayed. In such areas, sewage sludge compost could restore the soil's agronomic potential and stimulate plant succession processes.

Most of the studies dealing with compost effects on soil properties only take into account the organo-mineral A horizon compartment, without studying compost effects on humiferous episolumproperties. In this study, we followed the patterns of change in the litter, humus and organo-mineral A horizon compartments in a seven-year-old garrigue over two years after compost spreading. Three treatments were studied: control, 50 Mg.ha⁻¹ and 100 Mg.ha⁻¹ (Fresh Mass) of co-composted sewage sludge and green wastes.

After amendment, the major changes concern the humus and litter compartments, whereas the organo-mineral A horizon is slightly affected. The organo-mineral A horizon is enriched in exchangeable K, Mg and sporadically in P after amendment, slightly improving overall fertility, while the humus and litter compartments keep compost organic matter and its associated nutrients accumulated, showing the slow release of elements from the mature compost chosen. Compost amendment probably enhances the humus decomposition process,

first because it decreases this compartment's C/N ratio, and second because it decreases its Ni and Cr concentrations. In contrast to humus, compost should reduce the litter decomposition process, by increasing both its C/N ratio and pH, and by increasing concentrations of potentially toxic trace elements such as Cu, Zn, Ni, Cr, and Pb. Compost greatly enriched humus and litter in P, Cu, and Zn, but these element concentrations remained stable during the two years of experimentation, indicating probable low leaching down to groundwater. However, some Cd could have been leached down to groundwater.

Keywords: Mediterranean ecosystem, sewage sludge compost, litter, humus, trace elements, phosphorus, contamination, restoration.

Introduction

Both long dry summers and strong and dry winds characterize the Mediterranean climate, and favour recurrent forest fires (De Luis *et al.*, 2001 ; Scarascia-Mugnozza *et al.*, 2000). Fire consumes organic matter (above ground vegetation, litter, superficial soil layers) and its nutrients are either volatilized or deposited in the ash (Grogan *et al.*, 2000). Nutrients volatilized, especially N, are lost from the site. Those not absorbed by plants are prone to erosion or leaching to groundwater. Indeed, violent precipitation events are frequent in Mediterranean region (Scarascia-Mugnozza *et al.*, 2000) and induce soil impoverishment and thinning, when occurring after fire. Thus, the Mediterranean soils are often deficient in organic matter, nitrogen and phosphorus (Archibold, 1995), and have a low water availability (Le Houerou, 1973). The soils of the most degraded calcareous areas are often colonized by *Quercus coccifera* garrigues (Barbero, 1990). These evergreen stands might maintain the low-resource availability level of the sites they colonize (Aerts, 1995). Indeed, the sclerophyllous nature of evergreen leaves promotes gradual litterfall and slow rates of decomposition, thus moderating pulses of nutrients back into the soil and promoting tighter cycles of nutrient flow (Rundel, 1988). Thus, the installation of tree species, which develop under higher fertility level, is prevented, and these garrigues can be considered as a blocking stage of succession (Lepart and Escarre, 1983; Quézel and Médail, 2003).

Sewage sludge is a source of organic matter and plant nutrients (Brockway, 1983; Martinez *et al.*, 2003a), since it can improve soil physical and chemical properties (Caravaca *et al.*, 2002; McKay and Moffat, 2001). However, its use presents potential environmental risks, as heavy metal and organic contaminants accumulation in soils (Brockway, 1983), as well as the

discharge of nutrients, especially N and P, to surface and groundwater (Martinez *et al.*, 2003a). Composting could stabilize the organic matter concentration of sewage sludge and by this way decrease the risks of heavy metals and salts leaching (Garcia *et al.*, 1990; Planquart *et al.*, 1999). It is a biological process of aerobic decomposition that degrades labile organic matter to carbon dioxide, water vapour, ammonia, inorganic nutrients to obtain a stable organic material (compost) containing humic-like substances (Senesi, 1989). In addition, the use of other organic wastes with large C/N ratios (such as green wastes) in mixture with sewage sludge can reduce the rate of nitrogen mineralization that first decrease its leaching risks (McKay and Moffat, 2001) and second provide a durable release to plants.

Yet, compost amendment has been frequently shown to increase soil fertility (Caravaca *et al.*, 2002; Martinez *et al.*, 2003a), plant biomass (Guerrero *et al.*, 2001; Moreno-Peñaranda *et al.*, 2004) and plant nutrition (Moreno *et al.*, 1996). As Mediterranean soils are degraded, compost amendment could recreate favorable conditions for tree species occurrence by raising the fertility level of soils.

Therefore, we spread sewage sludge and green wastes compost on a degraded calcareous Mediterranean soil colonized by a 7-year old *Quercus coccifera* garrigue, and we followed the evolution of litter, humus and organo-mineral A horizon characteristics during the two years following amendment. Both above ground litter and humus are the sources of the nutrients accumulated in the uppermost layers of the soil (Santa Regina *et al.*, 2001). Above ground litter plays a fundamental role in the nutrient turnover of soil / plant system. It contributes to soil humus formation. Consequently, litter and humus are of primary importance in the nutrient budgets of Mediterranean ecosystems and we studied compost effects on these humiferous episolumcompartments, which differ from other studies that rather take into account organo-mineral A horizon compartment. Our aims were to (i) determine compost amendment effect on the dynamic of organo-mineral A horizon, humus and litter fertility parameters, (ii) follow the fluxes of compost elements through the three studied compartments, and (iii) study the eventual soil contamination by trace metals after amendment.

Material and methods

Study site and experimental design

The experiment was carried out on 6000 m² in the plateau of Arbois (Southern Provence, France; 5°18'6''E – 43°29'10''N in WSG-84 Norm) at 240 m above sea level and under Mediterranean climatic conditions. The soil was a silty-clayey chalky rendzina, with a high percentage of stones (77 %) and a low average depth (around 24 cm). The last fire occurred in June 1995 and the site was colonised by Mediterranean sclerophyllous vegetation, with a 70 % total cover; *Quercus coccifera* L. and *Brachypodium retusum* Pers. being the two dominant species. This natural vegetation belongs to the hoalm oak (*Quercus ilex* L.) succession series. Compost was surface applied in January 2002. It was put in a tipper-wagon pulled by a tractor, which went over each plot, step by step, on length lines. The experimental design was a complete randomised block of twelve plots of 500 m². Four plots did not receive any compost (D0=control), four received 50 Mg.ha⁻¹ (D50), and four 100 Mg.ha⁻¹ of fresh compost (D100).

Compost

The compost was produced by a local company (Biotechna, Ensues, Southern Provence) and is certified conform to the French norm on composts made with materials of water treatment origin (NF U 44-095, 2002). This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in swathes. The swathes were mixed several times in the next 6 months to promote organic matter humification. The final compost met the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals. Characteristics of soil before amendment and compost are shown in Table 1.

Field procedures

Organo-mineral A horizon and humiferous episolum were sampled thirteen times from March 2002, one month and a half after amendment, to March 2004, approximately two years after amendment. All humiferous episolum was collected on a 20 x 20 cm square. Then, all accessible organo-mineral A horizon down to bedrock was collected. Each analysed organo-mineral A horizon and humiferous episolum sample was a mix of 3 samples randomly collected on each 500-m² plot.

Table 1. Organo-mineral A horizon (0-24 cm: maximal depth; N=12) and compost (N=3) physicochemical characteristics. DM: Dry Matter. FM: Fresh Mass. D50: 50 Mg.ha⁻¹ of compost.

| Parameter | Soil | | Compost | | |
|--|----------------|---|---------------------------|---|--|
| | Mean (SE) | Allowed French limit value before sewage sludge amendment | Mean (SE) Fraction < 2 mm | Allowed French limit value (08/01/1998) | D50 calculated concentration increase in soil fraction < 2mm (%) |
| pH _{H2O} | 7.34 (0.008) | | 7.7 (0.05) | | |
| Density (DM, unsieved fraction) | 1.4 (0.09) | | 0.56 (0.03) | | |
| humidity (% FM) | | | 4.8 (0.29) | | |
| CEC (meq.100 g ⁻¹) | 23.12 (0.31) | | | | |
| total CaCO ₃ (%DM) | 4.17 (0.13) | | | | |
| OM (% DM) | 7.58 (0.12) | | 46.8 (2.74) | | 187 |
| total N (% DM) | 0.36 (0.005) | | 2.03 (0.03) | | 171 |
| C/N | 12.42 (0.09) | | 13.4 (0.78) | | |
| total P (% DM) | 0.037 (0.001) | | 3.24 (0.03) | | 2655 |
| exchangeable P ₂ O ₅ (ppm) | 53.4 (0.8) | | 5759 (18) | | 3272 |
| Soil exch / compost total K ₂ O(% DM) | 0.04 (0.0003) | | 0.77 (0.01) | | |
| Soil exch / compost total MgO (% DM) | 0.019 (0.0001) | | 0.81 (0.02) | | |
| Soil exch / compost total CaO(% DM) | 1.34 (0.009) | | 16.8 (0.17) | | |
| Copper (mg.kg ⁻¹ DM) | 19.8 (0.14) | 100 | 144.1 (0.84) | 1000 | 221 |
| Zinc (mg.kg ⁻¹ DM) | 78.2 (0.24) | 300 | 265.0 (5.49) | 3000 | 103 |
| Cadmium (mg.kg ⁻¹ DM) | 0.31 (0.002) | 2 | 0.8 (0.0) | 15 | 78 |
| Chrome (mg.kg ⁻¹ DM) | 67.3 (0.33) | 150 | 27.1 (0.65) | 1000 | 12 |
| Mercury (mg.kg ⁻¹ DM) | 0.06 (0.001) | 1 | 0.86 (0.06) | 10 | 435 |
| Nickel (mg.kg ⁻¹ DM) | 45.3 (0.17) | 50 | 16.5 (0.23) | 200 | 11 |
| Lead (mg.kg ⁻¹ DM) | 43.1 (0.26) | 100 | 57.3 (2.53) | 800 | 40 |

Table 2. Norms used for soil, humus and litter analysis.

| parameter | Soil and humus | litter |
|--|------------------|--------------------------------|
| pH _{H2O} | NF ISO 10390 | PRU 44172 |
| Cation exchange capacity | NF X 31-130 | |
| Total N | ISO 13878 | NF EN 13654-2 |
| Organic C | NF ISO 14235 | NF EN 13039 |
| Organic matter | 1,72 x Organic C | 1,72 x Organic C |
| Exchangeable K and Mg NH ₄ Ac | NF X 31-108 | |
| Total P ₂ O ₅ | NF EN ISO 11885 | |
| Exchangeable P ₂ O ₅ | NF EN ISO 11263 | NF EN ISO 11263 |
| Exchangeable Cu and Zn EDTA | NF X 31-120 | |
| Total Cd, Cr, Cu, Ni, Pb, Zn | NF EN ISO 11466 | |
| Total Hg | NF X31-151 | NF EN 12338 |
| Total Cd, Cr, Cu, Ni, Pb, Zn and P ₂ O ₅ | | NF EN 13650 NF EN ISO 11885 |

Laboratory procedures

Before analysis, samples of organo-mineral A horizon and humiferous episolum were 2-mm mesh sieved, and oven dried at 45°C to constant weight. The humiferous episolum fraction > 2 mm corresponded to litter compartment and contained compost pine barks and wood pieces, and coarse plant litter. The humiferous episolum fraction < 2 mm corresponded to humus compartment and contained humified organic matter and composted sewage sludge (granulometric definition). Humidity was measured by oven drying samples at 60°C during 3 days. Sample analyses are performed according to French norms (Table 2).

The $\text{pH}_{\text{H}_2\text{O}}$ was measured in distilled water (PHM 240 Radius méter). Cation exchange capacity (CEC) was measured by soil percolation with an ammonium acetate solution and organic C was determined using sulphuric-chromic oxidation and spectrophotometry (Cary 50 VARIAN). Exchangeable potassium (K) and magnesium (Mg) were 1M NH_4Ac extracted and analysed by atomic absorption spectrophotometry (FS220 VARIAN). Total nitrogen (N) was determined by dry combustion and thermic conductimetry (FP 428 LECO). Exchangeable P was determined in an sodium hydrogenocarbonate solution using spectrophotometry (Olsen *et al.*, 1954) (Cary 50 VARIAN). Cu and Zn exchangeable forms were extracted using EDTA and concentrations were assessed by atomic absorption spectrophotometry (VARIAN VISTA Radial). To measure total concentrations of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), phosphorus (P_2O_5), and zinc (Zn), samples were digested in *aqua regia* and analysed using plasma emission spectrophotometry (VARIAN VISTA Axial).

Statistical analyses

Two way ANOVA combined with Tukey test (Zar, 1984) were used to study compost rate and sampling date effects on the different soil, humus and litter parameters. If any interaction occurred between the two studied factors (compost, date), one way ANOVA were performed at each sampling date (2002: March, April, May, June, July, September, October, November, December; 2003: March, June, October; 2004: March) to study compost rate effect. Significant level was considered to be 95 %. The softwares Statgraphics plus (version 2.1: Statistical Graphics Corporation[®], Copyright 1994-1996) and Minitab[®] (release 13 for Windows, 2000, Minitab Inc., USA) were used.

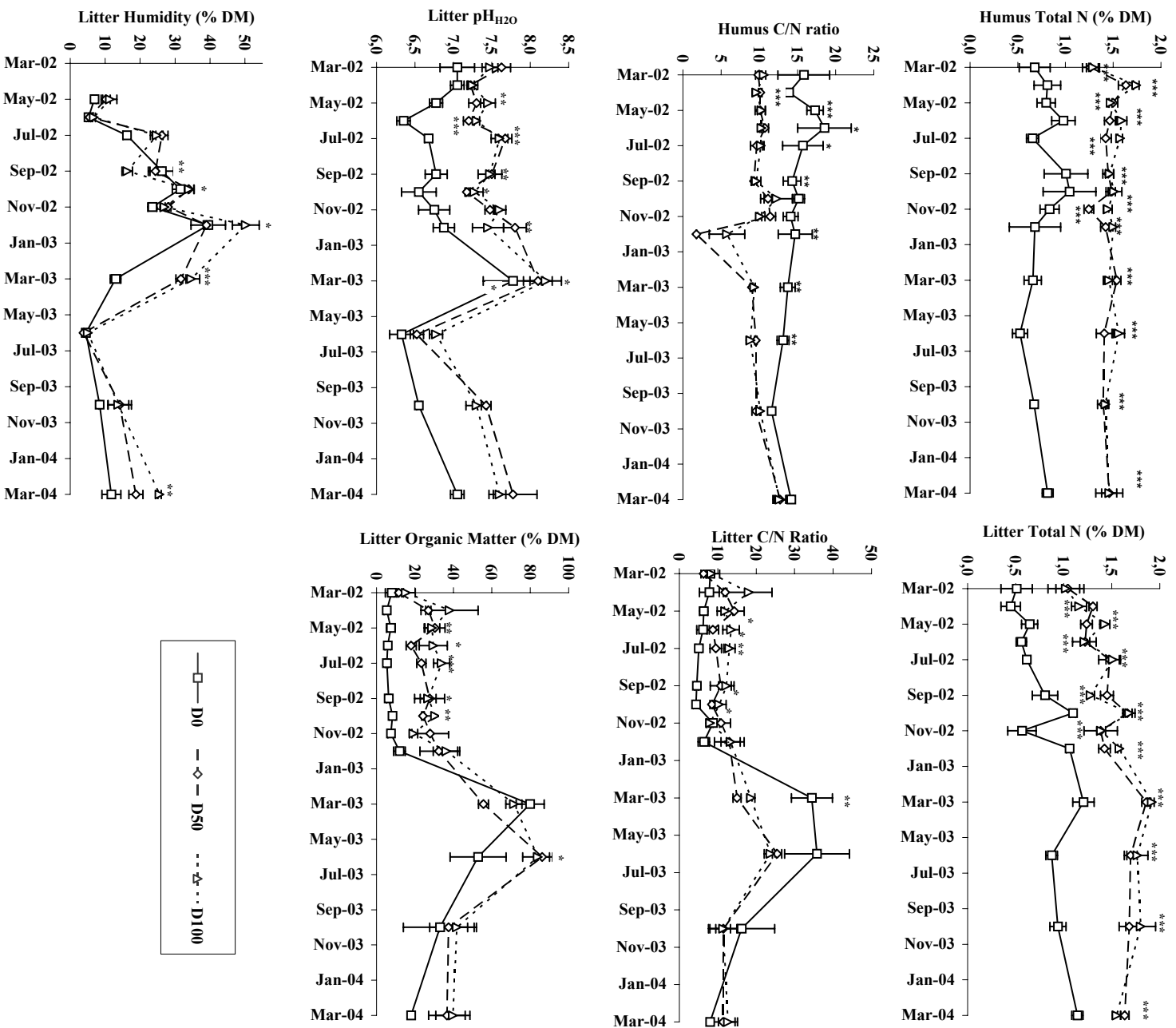


Figure 1. Total N (humus and litter), C / N ratio (humus and litter), OM (litter), and pH_{H2O} (litter), from March 2002 to March 2004, for D0 (control), D50 (50 Mg·ha⁻¹ of compost), and D100 (100 Mg·ha⁻¹ of compost) plots. Mean ± SE, N=4. ANOVA: *p<0.05; **p<0.01; ***p<0.001.

Results

Considering the numerous parameters studied, only the statistically significant results will be shown.

pH, humidity, OM, CEC, total N and C/N ratio (Figure 1)

The compost had a significant effect neither on organo-mineral A horizon pH, nor on its cationic exchange capacity and organic matter rate. Similarly, organo-mineral A horizon N concentration and C/N ratio were not affected by compost amendment during the two years following amendment, as well as this compartment and humus humidity.

In contrast, compost strongly and significantly increased the total N concentrations in both humus (two way ANOVA, $p < 0.0001$) and litter (two way ANOVA, $p < 0.0001$) compartments. The total N concentrations nearly doubled on plots amended at both rates compared to control, and this effect persisted two years after amendment (March 2004).

However, the compost had an opposite effect on the two latter compartments for the C/N ratio. This ratio increased significantly (one way ANOVA, May, June, July, September and October 2002, $0.05 > p > 0.01$) in the litter compartment of amended plots, while it decreased significantly in humus (one way ANOVA, April, May, June, July, September and December 2002, March and June 2003, $p < 0.05$). The second year (2003), the C/N ratio decrease in humus of amended plots disappeared in October 2003 and March 2004. Moreover, throughout 2003, the control litter C/N values strongly increased and the tendency was reversed, as the C/N ratio in control plot litter reached higher values than those measured on amended plots. In March 2004, amended and control plots showed similar litter C/N ratio values.

The same pattern was observed for litter organic matter rate, which was significantly higher overall (two way ANOVA, $p < 0.0001$) on amended plots during the experiment, and was sporadically exceeded by control values in March 2003. At this date, compost significantly improved litter humidity (one way ANOVA, $p < 0.0001$) on amended plots compared to control, both in July 2002, December 2002, and March 2004 (one way ANOVA, $p < 0.05$ for the 3 dates). Finally, the litter pH significantly increased (two way ANOVA, $p < 0.0001$) by approximately 0.5 pH unit for both compost rates in 2002, and this effect disappeared the second year.

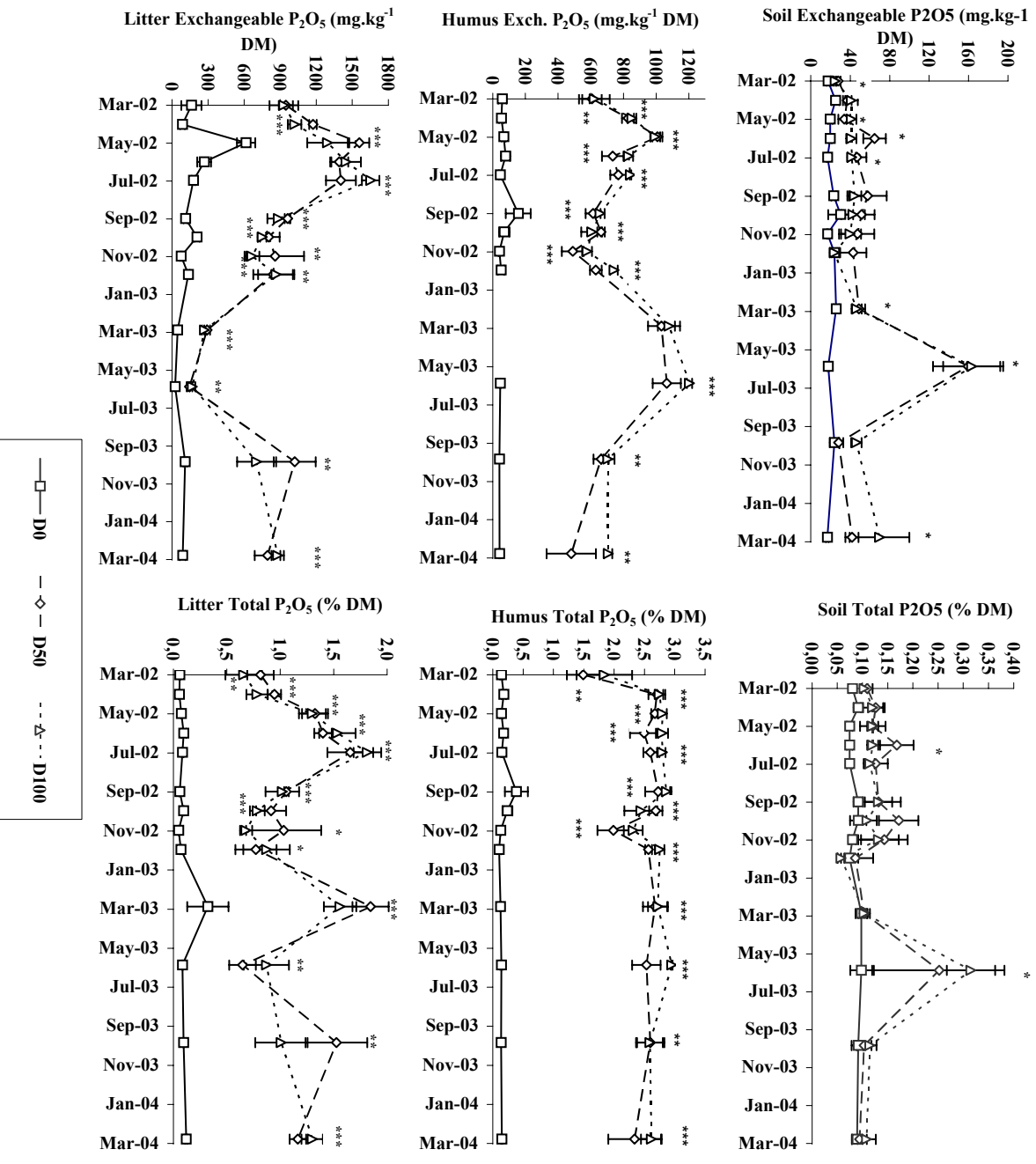


Figure 2. Phosphorus concentrations in the 3 studied compartments (organo-mineral A horizon, humus, litter), from March 2002 to March 2004, for D0 (control), D50 (50 Mg ha⁻¹ of compost), and D100 (100 Mg ha⁻¹ of compost) plots. Mean \pm SE, N=4. ANOVA: *p<0.05; **p<0.01; ***p<0.001.

Phosphorus (Figure 2)

Compost induced very strong increases in exchangeable and total P concentrations in humus and litter, while this increase was less marked in the organo-mineral A horizon. In the latter compartment, peaks of total and exchangeable P occurred on amended plots in June 2003. At this date, total and exchangeable P concentrations in the organo-mineral A horizon were significantly higher (one way ANOVA, $p < 0.05$ for total P, $p < 0.01$ for exchangeable P) than control in amended plots. However, compost effect on exchangeable P concentrations was more pronounced than for total P, as concentrations also significantly increased on amended plots at other dates than June (one way ANOVA, March, May, July 2002, March 2003, and March 2004, $p < 0.05$).

Total P concentration in humus and litter were 30 to 50 times significantly higher (humus: two way ANOVA, $p < 0.0001$; litter: one way ANOVA, all dates, $p < 0.01$) than control on amended plots over the whole experiment. The total P concentration was stable in humus (about 2.7 % of DM), whereas it showed seasonal variations in litter. The first year, maximum concentrations in litter were reached in spring and early summer (May, June, and July 2002). However, in 2003, total P concentrations were at a minimum in June, while they were twice as high in March and October.

Exchangeable P concentrations were also about 15, and between 7 and 8 times significantly higher than control on amended plots, respectively for humus (one way ANOVA, all dates except March 2003, $p < 0.01$) and litter (one way ANOVA, all dates, $p < 0.01$), over the whole experiment. However, the dynamics were different in the two compartments of amended plots. In March and June 2003, exchangeable P concentrations were highest for humus, whereas they were lowest for litter, being nearly similar to the control values.

Cations (Figure 3)

Compost had an overall significant improving effect on exchangeable K and Mg concentrations in both organo-mineral A horizon (two way ANOVA, $p = 0.04$ and $p = 0.01$, respectively for K and Mg) and humus (one way ANOVA for K, three dates, $p < 0.05$ and two way ANOVA for Mg, $p < 0.0001$). Exchangeable K was 3 times higher than control in humus of amended plots one and a half months after amendment (March 2003). This compost-linked increase in humus strongly decreased over time for both compost rates, but remained

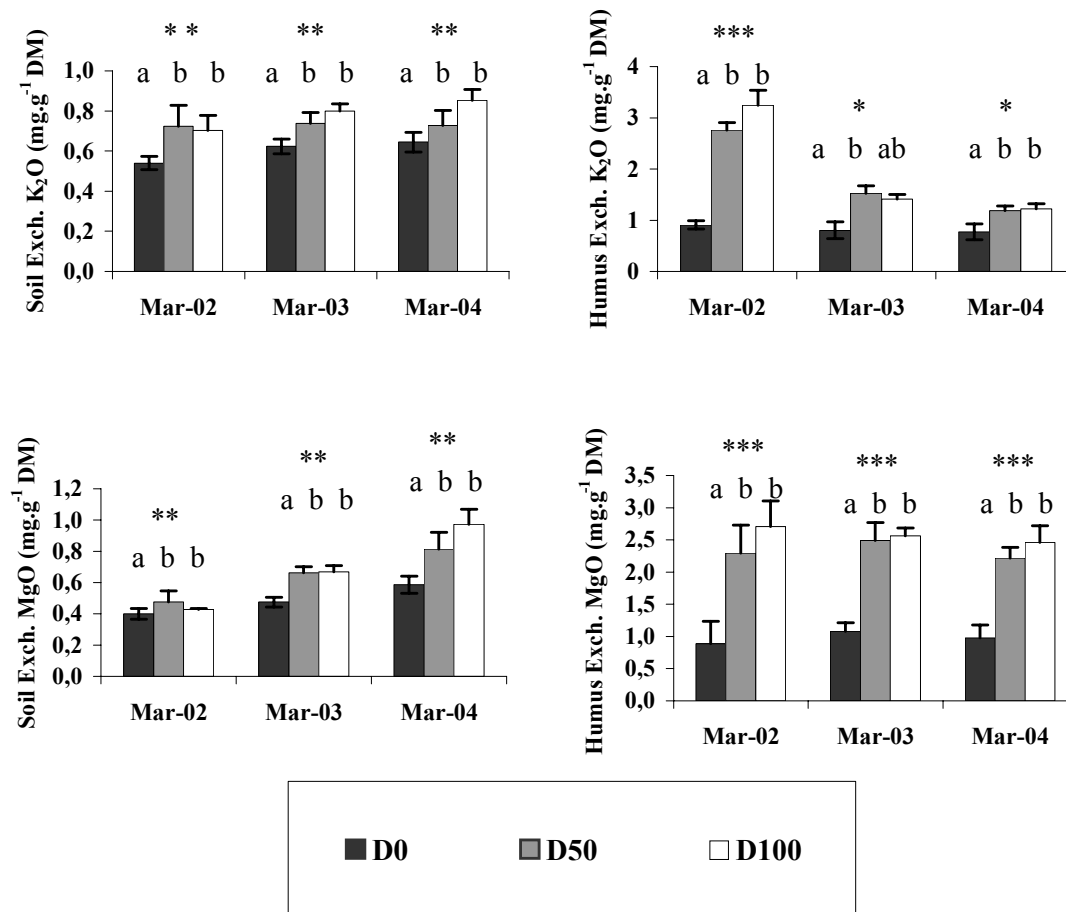


Figure 3. Potassium and Magnesium concentrations in organo-mineral A horizon and humus, in March 2002, 2003 and 2004, for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean \pm SE, N=4. ANOVA: *p<0.05; **p<0.01; ***p<0.001.

significant two years after amendment (March 2004). In the organo-mineral A horizon, the increase was slighter, as exchangeable K concentrations increased by 25 % on amended plots compared to control. However, the durability of this effect varied according to the compost rate, as exchangeable K concentration on D100 was still 25 % higher than control in March 2004, whereas D50 values were intermediate between control and D100.

Exchangeable Mg concentrations were significantly and durably improved in the humus compartment for the two compost rates. Exchangeable Mg concentrations were multiplied by a factor between 2 and 3 for the three dates on amended plots compared to control. The compost increasing effect on exchangeable Mg concentrations was lower in the organo-mineral A horizon compartment (+ 50 % on amended plots compared to control). In addition, this effect was very progressive and increased with time (two way ANOVA, $p < 0.0001$). In March 2004, exchangeable Mg concentrations were the highest on D100.

Trace metals (Table 3, Figure 4, and Figure 5)

The organo-mineral A horizon was overall not enriched in trace metals after compost amendment, and during the two years of experimentation. Only exchangeable Cu concentrations slightly and significantly increased (two way ANOVA, $p = 0.048$) on D50 compared to control, for the three sampling dates (March 2002, 2003, and 2004) (Table 2).

In the humus compartment (Figure 4), total Cd, Cu and Zn respectively increased by a factor of around 2, 4, and 3 on amended plots (two way ANOVA, $p = 0.0003$, $p < 0.0001$, and $p < 0.0001$, respectively for Cd, Cu, and Zn) compared to control. At the end of the experiment, these compost increasing effects remained for Cu and Zn at both rates, while total Cd concentration had decreased to reach control concentration value on D50, and remained higher on D100. Compost had no significant effect on humus total Pb concentrations.

Total Ni and Cr concentrations significantly decreased (two way ANOVA, $p < 0.0001$ for both elements) in humus of amended plots compared to control. Concentrations of these elements were 25 % , and 30 to 50 % lower on plots amended at both rates, respectively for Cr and Ni, compared to control.

In the litter compartment (Figure 5), as in humus, compost significantly increased total Cd, Cu, and Zn concentrations (one way ANOVA, March 2002: $p < 0.01$, March 2003: $p < 0.0001$, and March 2004: $p < 0.01$, for the 3 elements) compared to control, for the 3 sampling dates. The increases were of the same order values as in humus compartment for Cd and Zn, but were higher for Cu (5 to 10 times higher in litter against 4 times higher in humus). The

Table 3. Trace metals concentrations in organo-mineral A horizon in March 2002, 2003 and 2004 for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean (SE), N=4. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (Tukey test).

| | rate | Total Cd mg.kg ⁻¹ DM | Total Cr mg.kg ⁻¹ DM | Total Cu mg.kg ⁻¹ DM | Total Ni mg.kg ⁻¹ DM | Total Pb mg.kg ⁻¹ DM | Total Zn mg.kg ⁻¹ DM | Exch Cu mg.kg ⁻¹ DM | Exch Zn mg.kg ⁻¹ DM |
|------------|------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|-----------------------------------|-----------------------------------|
| March 2002 | D0 | 0,33 (0,03) ^a | 60 (2,4) ^a | 19,3 (1,4) ^a | 41 (1,7) ^a | 44 (3,0) ^a | 79 (3,6) ^a | 1,6 (0,1) ^a | 2,7 (0,4) ^a |
| | D50 | 0,33 (0,03) ^a | 61 (1,5) ^a | 21,2 (1,2) ^a | 42 (1,1) ^a | 50 (4,0) ^a | 87 (5,9) ^a | 1,8 (0,1) ^b | 4,0 (1,1) ^a |
| | D100 | 0,3 (0,04) ^a | 63 (1,8) ^a | 21,2 (1,3) ^a | 43 (1,2) ^a | 42 (1,8) ^a | 122 (44,1) ^a | 1,7 (0,1) ^{ab} | 7,4 (4,5) ^a |
| March 2003 | D0 | 0,25 (0,03) ^a | 63 (2,8) ^a | 20,4 (1,3) ^a | 42 (2,2) ^a | 49 (2,0) ^a | 83 (4,1) ^a | 2,1 (0,1) ^a | 4,5 (0,5) ^a |
| | D50 | 0,25 (0,03) ^a | 64 (1,6) ^a | 21,0 (0,8) ^a | 45 (0,8) ^a | 50 (2,2) ^a | 85 (2,1) ^a | 2,4 (0,1) ^b | 4,8 (0,4) ^a |
| | D100 | 0,23 (0,03) ^a | 68 (2,1) ^a | 21,7 (1,0) ^a | 46 (0,1) ^a | 49 (1,2) ^a | 84 (2,1) ^a | 2,3 (0,1) ^{ab} | 5,0 (0,5) ^a |
| March 2004 | D0 | 0,15 (0,03) ^a | 71 (3,3) ^a | 20,9 (1,1) ^a | 48 (2,3) ^a | 43 (1,2) ^a | 78 (3,4) ^a | 2,0 (0,1) ^a | 3,9 (0,4) ^a |
| | D50 | 0,18 (0,03) ^a | 67 (1,8) ^a | 20,3 (0,5) ^a | 47 (1,6) ^a | 57 (7,7) ^a | 81 (4,0) ^a | 2,2 (0,1) ^b | 5,4 (0,5) ^a |
| | D100 | 0,15 (0,03) ^a | 68 (1,1) ^a | 20,4 (1,5) ^a | 47 (0,5) ^a | 42 (3,7) ^a | 76 (3,1) ^a | 2,2 (0,3) ^{ab} | 5,3 (1,2) ^a |

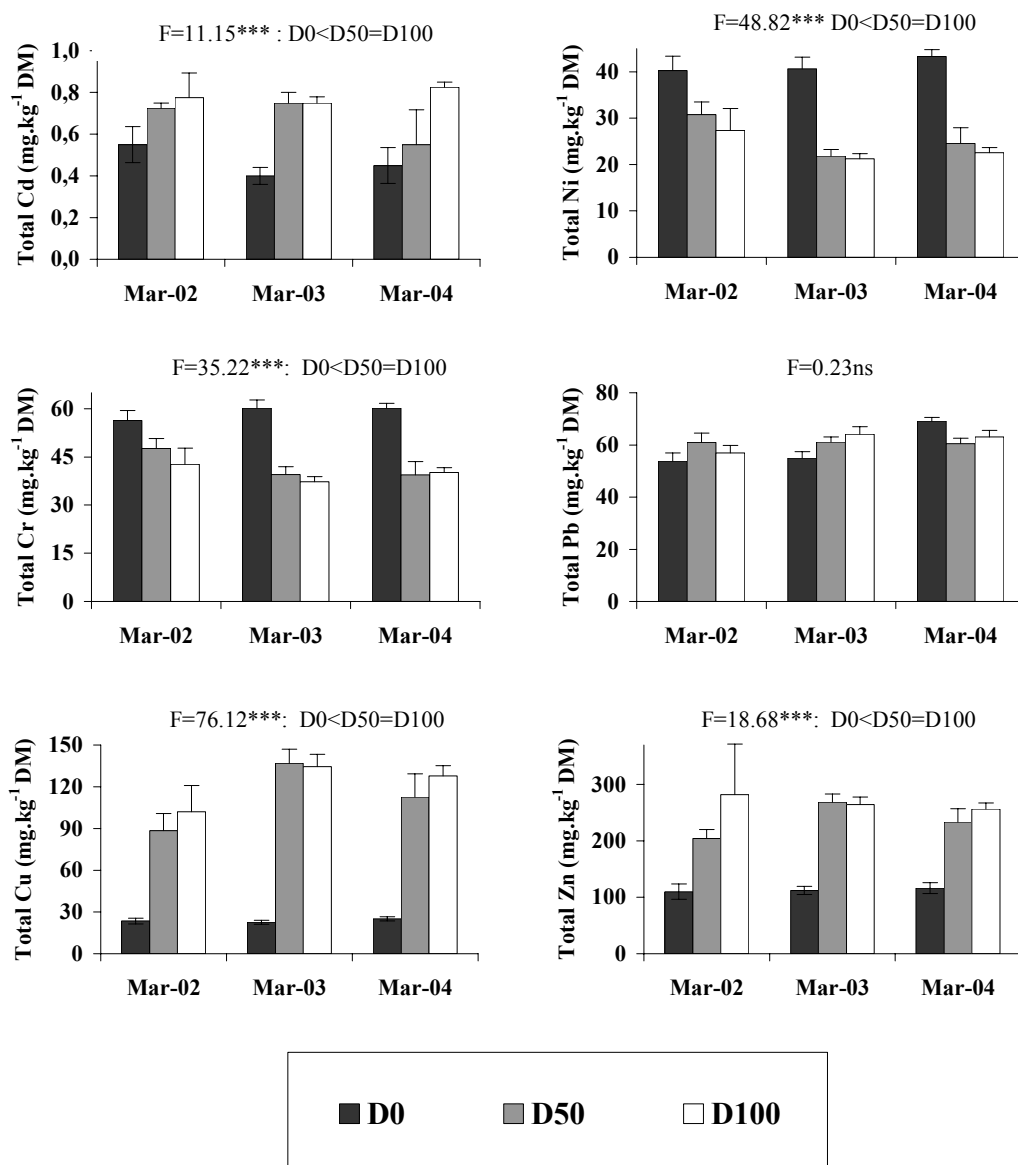


Figure 4. Trace metal concentrations in humus, from March 2002 to March 2004, for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean \pm SE, N=4. ANOVA: * p <0.05; ** p <0.01; *** p <0.001.

maximum concentration values were reached in March 2003 on amended plots for the 3 elements. However, in contrast to humus, total Cr, Ni, and Pb concentrations sporadically reached (March 2003: one way ANOVA, $p < 0.001$ for the 3 elements) higher values than control in the litter of amended plots, especially for D50 rate. Concentrations of these elements approximately doubled on D50 compared to control in March 2003. Moreover, the control concentrations of these 3 elements in litter increased in March 2004.

Discussion

Compost fertilization effects

As sewage sludge contains large amounts of N, amendment greatly enriches humus and litter in this element. However, the compost used has been subjected to a 6 months maturation phase that led to a very stable and humified product, and limited the organo-mineral A horizon N enrichment by percolation.

The sewage sludge entering compost composition comes from a sewage plant that applies a dephosphatation process to sewage. Consequently, P is concentrated in the residual sludge, leading to a P-rich compost that strongly and durably increases total and exchangeable P concentrations in both humus and litter compartments. In our experiment, and in contrast to other authors (Martinez *et al.*, 2003a), this element does not seem to be very mobile down to soil, as P concentrations poorly increase in the organo-mineral A horizon of amended plots compared to the high inputs of P by compost in the humus and litter compartments. In Ca-rich humus, P may form inorganic calcium phosphate (Khanna and Ulrich, 1984), which decreases its solubility.

Archibold (1995) notes that N and P may be the most limiting elements for plant growth in Mediterranean ecosystems, and both elements have critical importance in plant nutrition (Martin-Prével *et al.*, 1984; Rundel, 1988). Despite low enrichment of the organo-mineral A horizon in N and P, the compost mulch should have greatly improved vegetation nutrition. Most feeding roots can directly dip into litter and humus nutrient reserves (Miller, 1984).

Compost increases exchangeable K and Mg concentrations in humus, inducing the enrichment of the organo-mineral A horizon in these two elements, probably by percolation. Likewise, several authors noted an improvement of soil fertility after compost amendment (Guerrero *et al.*,

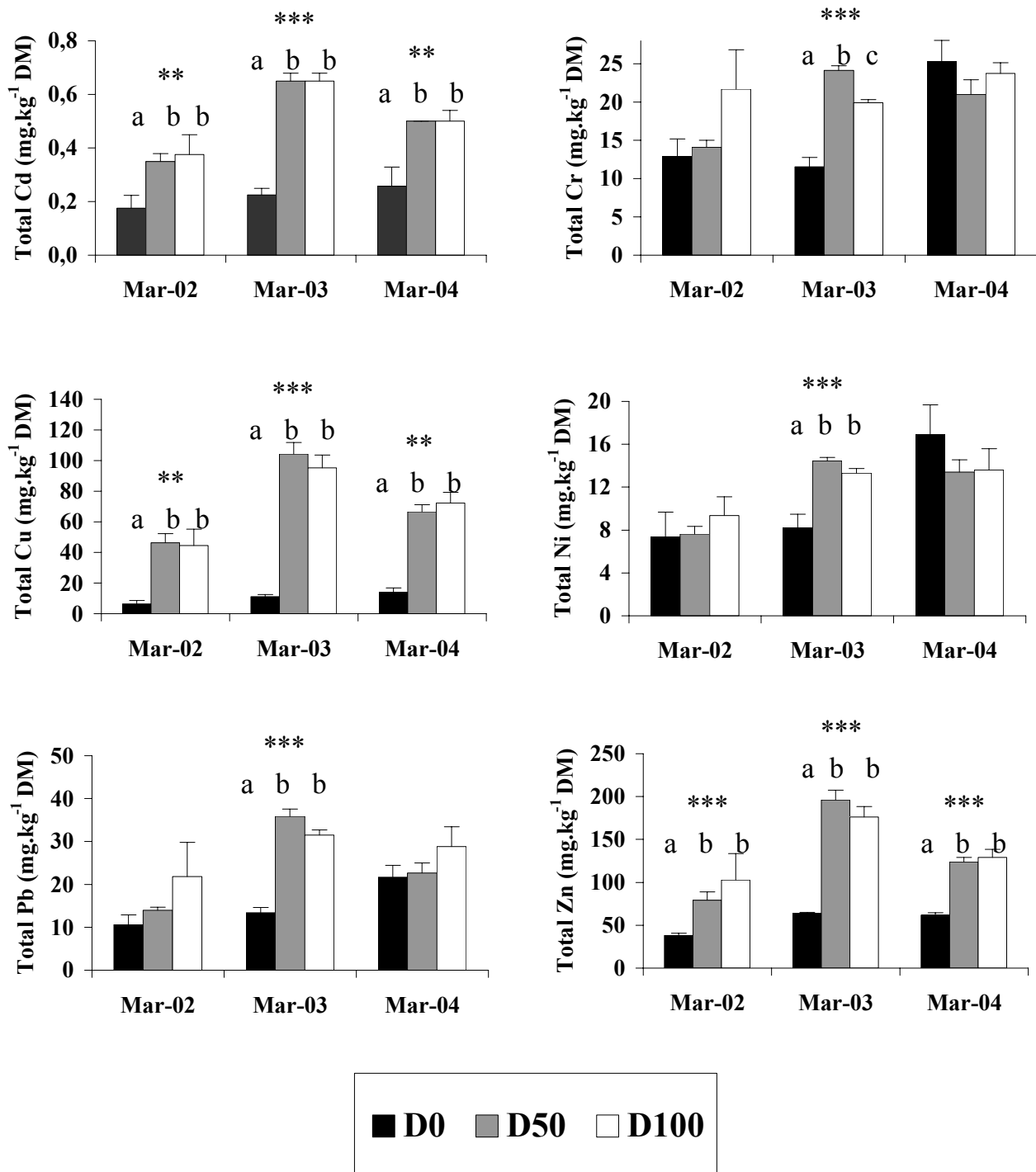


Figure 5. Trace metal concentrations in litter, from March 2002 to March 2004, for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean \pm SE, N=4. ANOVA: *p<0.05; **p<0.01; ***p<0.001.

2001; Korboulewsky *et al.*, 2002; Martinez *et al.*, 2003a). Exchangeable K concentration decreases over time in humus, reflecting a progressive depletion of this compartment's K reserve. On the other hand, the compost's improving effect on humus Mg reserve is more durable than for K, whereas the compost initial inputs for these two elements are roughly similar. As the compost greatly increases humus P concentration, the formation of relatively insoluble magnesium phosphate which is resistant to leaching (Embleton, 1966) should give moderate Mg percolation down to organo-mineral A horizon, improving the durability of its fertilization effect, and explaining the very progressive organo-mineral A horizon enrichment in this element.

Compost effect on humiferous episolum decomposability

Amendment greatly enriches humus in N, and consequently the C/N ratio decreases in amended plots during the first year. However, the compost's decreasing effect on humus C/N ratio disappears at the end of the experiment, probably due to the depletion of compost supplied N, as several authors have shown the durability of the compost's fertilization effects to be limited (Guerrero *et al.*, 2001; Martinez *et al.*, 2003a). In the litter compartment, compost amendment introduces pine bark and green waste that have a high C/N ratio, and consequently the C/N ratio value increases despite simultaneous N enrichment. As C/N ratio and litter decomposability have been shown to follow an inverse linear relationship (Gosz, 1984), the amendment should reduce the litter decomposition process, whereas it should enhance the humus decomposition process. Furthermore, compost increases litter pH, that could promote bacteria, whereas it could limit fungi in the microbial community (Borken *et al.*, 2002). Yet fungi are able to decompose and assimilate refractory compounds such as polyphenols (lignin or tannins) (Criquet, 1999), although bacteria are not thought to be before the leaf material has been partially broken down and decomposed by fungi (Jensen, 1974). Thus, the decomposition of Mediterranean garrigue litter, containing leaves rich in decomposition inhibiting molecules such as polyphenols (Gershenson, 1984), should slow down after compost amendment. However, in March 2003, the compost increases litter humidity, and consequently improves this compartment's mineralization by microorganisms, leading to lower organic matter accumulation on amended plots compared to control. In Mediterranean ecosystems, microorganisms's decomposition activity is frequently reduced by the too low availability of water (Criquet *et al.*, 2002).

Compost amendment increases total Cu and Zn concentrations in both humus and litter, due to the compost richness in these two elements compared to the experimental soil. These increases should deplete decomposition process in the humus and litter compartments, because the total concentrations reached on amended plots are higher than concentrations reported to have toxic effects on microbial activity in soil, especially for Zn (Kabata-Pendias and Pendias, 1992).

Moreover, the experimental site had relatively high Cr, Ni and Pb concentrations before amendment, which is consistent with studies that assessed the contamination of French forest soils by trace elements (De Nicola *et al.*, 2003; Hernandez *et al.*, 2003). The origins of this contamination are assumed to be atmospheric, as the area receives significant acid atmospheric pollution (AIRMARAIX, 1999). As the compost is less contaminated than the control humus in Cr and Ni, its spread dilutes concentrations of these elements in humus, and probably enhances its decomposition process. Cr and Ni are toxic to microorganisms (Moreno *et al.*, 2003; Shi *et al.*, 2002), especially Ni that is toxic at lower levels than other trace metals (Kabata-Pendias and Pendias, 1992). However, compost is an extra source of heavy metals, even if low contaminated, and further changes in physicochemical soil conditions could induce some remobilization of these potentially toxic elements (Planquart *et al.*, 1999).

Even if the experimental site is contaminated before amendment, Pb and Cr are known to have low availability to plants and are absorbed at very low rates (Kabata-Pendias and Pendias, 1992). Thus, plants act as filters for these accumulated elements in soils, and consequently provide a very low level of contaminated litter in these elements. Moreover, even if Ni is reported to be more easily absorbed by plants, the calcareous soil of the experimental site should strongly decrease its availability (Kabata-Pendias and Pendias, 1992). Thus, the increase of Pb, Ni and Cr concentrations in litter compartment should be due to compost spreading rather than plant absorption of trace metals, even if the compost used has low concentrations in these 3 elements.

Interaction between compost and drought

The year 2003 was particularly dry in Southern France, with very low rainfall (24.4 mm) from May to September. In June 2003 (early summer), litter organic matter concentration reached very high values (80 %) on amended plots, probably due to the compost positive effect on

plant biomass production (Guerrero *et al.*, 2001; Moreno-Peñaranda *et al.*, 2004). In addition, June is the month with maximum recorded litterfall in Mediterranean ecosystems (Floret *et al.*, 1989) and higher biomass production implies higher litter production. However, fertilization has been shown to increase plant sensitivity to drought by increasing the size of transpiring shoots (Van Den Driessche, 1984). Consequently, compost could also have induced a higher plant defoliation rate under drought stress on amended plots, leading to an increase in fresh litter production. Moreover, the litter compartment constitutes the most superficial layer and is highly prone to desiccation under drought conditions. This could explain higher values of litter OM rates and C/N ratios obtained in the drought year 2003, due to the probable decrease of microbial activity.

In June 2003, P concentrations were maximum in the organo-mineral A horizon of amended plots, for total and exchangeable forms. The severe drought during this period probably decreased exchangeable P absorption by plants and consequently accumulation of this element occurred in the organo-mineral A horizon. However, drought induced higher plant mortality (unpublished data) and organo-mineral A horizon enrichment in dead roots. This fresh organic matter for decomposition could have stimulated the organo-mineral A horizon microflora, leading to higher P capture (increase of total P) and to pH decrease that could enhance calcium phosphate solubilization (increase of exchangeable P). Moreover, as fresh litter has a generally lower total P concentration than decomposed litter (Chauvet, 1987), it dilutes the total P of the litter compartment. In addition, low humidity induced very low P solubilization to exchangeable forms in litter in June 2003.

Finally, we have observed that the drought of the year 2003, by increasing plant stress level, increased some plant absorption of Pb, Ni and Cr (*Cistus albidus* L., unpublished data), leading to greater richness of the litter produced in March 2004 in these three elements, especially on control.

Compost pollution effects

Both compost maturity and site richness in calcium carbonate prevented P from leaching to groundwater, and organo-mineral A horizon contamination by total trace elements, despite high inputs of these elements in humus and litter after compost amendment. However, exchangeable Cu and P concentrations increased in the organo-mineral A horizon, and these elements had probably been leached down from litter and humus. These increases are very

slight and should not induce groundwater contamination. With regard to Cd, it seems that this element was leached to groundwater between March 2003 and March 2004 on D50 after humus enrichment, as its concentration decreased in March 2004 and as no organo-mineral A horizon contamination occurred on these plots. Among the other trace metals and whatever the soil characteristics, Cd is known to be most mobile ([Kabata-Pendias and Pendias, 1992](#)).

Conclusion

Compost spreading rather induces modifications of organic superficial layers (humus and litter), and has a slight effect on organo-mineral A horizon quality and contamination. Moreover, the compost effect is very different according to the compartment considered. Fertilization effects are marked in humus, and to a lesser degree in the organo-mineral A horizon. In contrast, compost should have rather depreciative effects on the decomposition process of litter compartment. However, organic matter and P accumulation due to the 2003 drought clearly indicates that the ecosystem functioning in the Mediterranean region is mostly influenced by climatic factors, especially by water availability. Groundwater pollution risks were low overall, but the persistence of contaminants such as trace elements in the system for long periods means that there is a need for longer time-scale surveys, especially in the case of mature compost that slowly decomposes.

Aknowledgements

This research was commissioned by the Conseil Général des Bouches-du-Rhône (France), and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the Rhône-Méditerranée-Corse French Water Agency.

Résultats

L'apport de compost modifie surtout les couches organiques superficielles du sol (humus et litière) et a peu d'effet sur l'horizon minéral. Les fractions humus et litière sont enrichies en N et surtout en P, provenant des boues d'épuration qui entrent dans la composition du compost. Cependant, la maturité du compost et la richesse du site en carbonate de calcium ont limité la percolation vers l'horizon minéral de ces éléments, et à plus forte raison les risques de pollution des eaux souterraines.

L'humus et la litière sont également enrichis en K et Mg après amendement, ainsi que l'horizon minéral, ce qui indique une plus grande mobilité de ces éléments par rapport au P. On remarque un épuisement de la réserve en K de l'humus sur parcelles amendées au bout de deux ans.

Le rapport C/N évolue de manière opposée dans les fractions litière et humus après amendement. Il augmente dans la litière, suite à l'apport par le compost d'écorces de pin à C/N élevé, ce qui peut avoir un effet dépréciateur sur le processus de décomposition de cette fraction. Ce possible effet limitant du compost sur la décomposition est renforcé par la diminution du pH de la litière sur parcelles amendées, qui pourrait modifier l'équilibre de la communauté microbienne en favorisant les bactéries au détriment des champignons. A l'opposé, le C/N de l'humus diminue après amendement, ce qui peut stimuler le processus de décomposition.

Par ailleurs, l'apport de compost augmente les teneurs en Cu et Zn de l'humus et la litière, éléments à potentiel effet toxique sur les microorganismes décomposeurs. Il semble que le Cu a percolé vers l'horizon minéral puisque la concentration de cette fraction en Cu échangeable augmente après épandage. Cependant, cette augmentation est relativement faible (10 à 15 %) et n'induit vraisemblablement pas de risque de pollution des eaux.

Par contre, il semblerait qu'un lessivage de Cd de l'humus à D50 s'est produit la deuxième année d'expérimentation vers les eaux de ruissellement ou souterraines. Concernant le Ni, le Cr, et le Pb, l'amendement a enrichi la litière en ces trois éléments potentiellement toxiques pour les microorganismes décomposeurs. Au contraire le compost peu contaminé en Cr et Ni a un effet de dilution à court terme (deux ans) sur l'humus du site expérimental fort concentré en ces deux éléments. Cet effet de dilution ne doit pas masquer le fait que l'apport, même limité en Cr et Ni, enrichit le site en éléments toxiques.

Enfin, la sécheresse de 2003 a induit une accumulation de matière organique dans la litière et de P dans l'horizon minéral, ce qui confirme le fait que le fonctionnement des écosystèmes

méditerranéens est préférentiellement influencé par les facteurs climatiques, notamment la disponibilité en eau, en comparaison des effets fertilisants du compost.

Chapitre 2

REPONSES NUTRITIONNELLES DES PLANTES A UN AMENDEMENT ORGANIQUE DANS UNE GARRIGUE MEDITERRANEENNE

Les concentrations en nutriments et éléments trace ont été suivies pendant deux années après amendement dans quatre espèces majoritaires de la garrigue : *Quercus coccifera*, *Brachypodium retusum*, *Ulex parviflorus* et *Cistus albidus*.

L'objectif était d'étudier la réactivité de ces différentes espèces de plantes, adaptées à un habitat à faible niveau de ressources, à une augmentation de la ressource en nutriments, ceci en relation avec leurs traits fonctionnels. De plus, cet article discute des conséquences possibles de l'amendement sur les cycles des éléments au sein du système sol / plante de la garrigue.

Plant nutritional responses to organic amendment in a Mediterranean post-fire garrigue.

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Article soumis le 21 octobre 2004 à Functional Ecology

Abstract

1. Mediterranean soils often have low organic matter and nutrient content due to recurrent forest fires and erosion. These degraded areas are colonized by evergreen and sclerophyllous plant communities that are adapted to low resource availability. In these ecosystems, what would be the plant reactions to an increase of resource availability ?

2. We followed the patterns of change in plant nutrient uptake in a seven-year-old garrigue in Southern Provence (France) during two years after organic amendment. Three treatments were studied: control, 50 Mg.ha⁻¹ and 100 Mg.ha⁻¹ of fresh co-composted sewage sludge and green wastes.

3. The amendment effect was greatest for P, and also improved N and cation nutrition.

4. Two factors seemed to control the compost improving effect on plant nutrition. First, drought globally decreased the compost effects, which shows that it is the main factor regulating plant activity in Mediterranean ecosystems. Secondly, the compost effect varied according to the plant's biological and physiological characteristics.

5. The garrigue vegetation showed temporal complementarity for nutrient recycling, according to the deepness of root system and to the leaf traits.

6. A complementary resource use was evidenced on the basis of three main types of plant nutrition strategy, *i.e.* accumulation, stress resistance and opportunism. This resource use pattern could be generalized to similar vegetation in Mediterranean-type areas of the world.

7. In conclusion, nutrients from the organic amendment were efficiently put in circulation by the major plant species of the garrigue. This shows that even if these species are adapted to low-resource environment, they are sensitive to an increase in resource level, and suggests that nutrient impoverishment after recurrent fire should be the major limiting factor of vegetation dynamic processes.

Keywords: fertilization, nutrient cycling, *Quercus coccifera* L., *Brachypodium retusum* Pers., *Cistus albidus* L.

Introduction

The Mediterranean climate is characterized by long dry summers, as well as frequent strong and dry winds. Both parameters favour recurrent forest fires (Barbero *et al.* 1987; Scarascia-Mugnozza *et al.* 2000; De Luis *et al.* 2001). Fire consumes organic matter (above ground vegetation, litter, superficial soil layers) and nutrients are either volatilized or deposited in the ash (Grogan, Bruns & Chapin 2000). Nutrients volatilized, especially N, or not immediately absorbed by plants are lost from the site. Those not immediately absorbed are taken away by erosion or leached into the groundwater. Fires eliminate plant cover and leave the soil unprotected against the impact of raindrops (De Luis *et al.* 2001). As Mediterranean region is characterized by violent precipitation events (Scarascia-Mugnozza *et al.* 2000), the continual removal of weathered rock material has resulted in rather thin, infertile soils, which are often deficient in organic matter, nitrogen and phosphorus (Le Houerou 1973).

Consequently, evergreen, sclerophyllous shrubs and trees are dominant in the five Mediterranean-type ecosystems of the world (Rundel 1988). Indeed, the evergreen habit is an adaptation to habitats with low nutrient availability (Aerts 1995), and sclerophylly is a drought adaptation (Rundel 1988). Furthermore, there might be a positive feedback between evergreenness and low nutrient availability (Aerts 1995). There is a tight relationships between soil and vegetation in ecosystem dynamics, originating from continuous fluxes of elements, chemicals and energy (Gimeno-Garcia, Andreu & Rubio 2001). The sclerophyllous nature of evergreen leaves promotes gradual litterfall and slow rates of decomposition, thus moderating pulses of nutrients back into the soil and promoting tighter cycles of nutrient flow (Rundel 1988).

Moreover, calcareous soils are known to limit plant absorption of some macro- and micro-nutrients. Indeed, the fixation of K^+ and NH_4^+ on illitic clay mineral particles in calcareous

soils limit their availability for plant growth (Khanna & Ulrich 1984). Similarly, Bingham (1966) reports that phosphorus in calcareous soils is not readily available to many plants, even though the soil total content is high. Finally, the alkalinity and buffering effects of the calcium carbonate decrease the availability of micronutrients, such as Mn, Fe, Zn, Cu and B (Chapman 1966), as they are fixed in relatively insoluble compounds (Larcher 1983).

In calcareous Provence, degraded soils prone to recurrent fires are colonized by *Quercus coccifera* garrigues (Trabaud 1987; Barbero 1990), which has been considered as a blocking stage of succession (Lepart & Escarré 1983; Quézel & Médail 2003). Indeed, this evergreen and sclerophyllous resprouting species is not dying out after fire, and as it has a quite long life, it forms relatively stable communities (Keeley 1986). In such plant communities, plant competition for limiting resources may be an important process determining which species are dominant (Tilman 1984). Changes in nutrient supply can strongly affect competition between plant species and the resulting species composition of plant community (Tilman 1984). In nutrient-poor habitats, an increase in nutrient disponibility leads to low-productive species with low biomass loss rates being replaced by highly productive species with high biomass loss rates (Aerts & Van Der Peijl 1993; Berendse 1998). Furthermore, where one or more nutrients are limiting, increasing their availability can increase cycling rates and C fixation, as increasing nutrient availability changes the litter quality, causing increased decomposition and maintaining higher nutrient availability (Gosz 1984). Thus, a nutrient supply in *Quercus coccifera* garrigue, leading to an increase of resource disponibility, could modify the dominance relationships between plant species.

Yet, compost amendment has been frequently shown to increase soil fertility (Caravaca *et al.* 2002; Martinez *et al.* 2003a), plant biomass (Guerrero *et al.* 2001) and plant nutrition (Moreno *et al.* 1996; Moreno-Peñaranda, Lloret & Alcañiz 2004). In contrast to inorganic fertilizers, sewage sludge and greenwastes compost is rich in humified organic matter, and can be used as a slow-release source of nutrients. But if the use of sewage sludge compost seems to be an attractive way to enhance soil potentialities and an alternative to sewage sludge landfill disposal, it presents potential environmental risks. Indeed, it can induce trace metal accumulation in soils (Brockway 1983), and consequently plant contamination. However, could a compost amendment increase nutrient absorption in a Mediterranean garrigue and decrease the dominance of low-resource adapted species, such as *Quercus coccifera*, and by this way favour progressive plant succession ?

We followed the nutrient and trace element concentrations in different species during the two years following amendment in a *Quercus coccifera* garrigue. Our aim was to study the reactivity of the different plant species infested to low resource habitats to an increase in nutrient supply in relation with their life-traits. In addition, the hypothetical consequences of amendment on element cycling in garrigue soil / plant system are discussed.

Material and methods

Study site and experimental design

The experiment was carried out on 6000 m² in the plateau of Arbois (Southern Provence, France; 5°18'6''E – 43°29'10''N in WSG-84 Norm) at 240 m above sea level and under Mediterranean climatic conditions. The soil was a silty-clayey chalky rendzina, with a high percentage of stones (77 %) and a low average depth (24 cm). The last fire occurred in June 1995 and the site is colonized by Mediterranean sclerophyllous vegetation, with a 70 % mean cover. *Quercus coccifera* L. and *Brachypodium retusum* Pers. are the two dominant species, and the vegetation belongs to the hoalm oak (*Quercus ilex* L.) succession series.

Compost was surface applied in January 2002. It was put in a tipper-wagon pulled by a tractor, which went over each plot, step by step, on length lines. The experimental design was a complete randomised block of twelve plots of 500 m². Four plots did not receive any compost (D0=control), four received 50 Mg.ha⁻¹ (D50), and four 100 Mg.ha⁻¹ of fresh compost (D100).

Compost

The compost was produced by a local company (Biotechna, Ensues, Southern Provence) and is certified conform to the French norm on composts made with materials of water treatment origin (NF U 44-095 2002). This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in swathes. The swathes were mixed several times in the next 6 months to promote organic matter humification. The final compost met the French legal standards for pathogenic

Table 1. Organo-mineral A horizon (0-24 cm: maximal depth; N=12) and compost (N=3) physico-chemical characteristics. DM: dry matter. FM: Fresh Mass.

| Parameter | Soil | | Compost | | |
|--|----------------|---|---------------------------|---|--|
| | Mean (SE) | Allowed French limit value before sewage sludge amendment | Mean (SE) Fraction < 2 mm | Allowed French limit value (08/01/1998) | D50 calculated concentration increase in soil fraction < 2mm (%) |
| pH _{H2O} | 7.34 (0.008) | | 7.7 (0.05) | | |
| humidity (% FM) | | | 4.8 (0.29) | | |
| CEC (meq.100 g ⁻¹) | 23.12 (0.31) | | | | |
| total Calcareous (%DM) | 4.17 (0.13) | | | | |
| OM (% DM) | 7.58 (0.12) | | 46.8 (2.74) | | 187 |
| total N (% DM) | 0.36 (0.005) | | 2.03 (0.03) | | 171 |
| C/N | 12.42 (0.09) | | 13.4 (0.78) | | |
| total P (% DM) | 0.037 (0.001) | | 3.24 (0.03) | | 2655 |
| exchangeable P (ppm) | 23.3 (0.35) | | 2514.8 (7.82) | | 3272 |
| Soil exch / compost total K (% DM) | 0.02 (0.0001) | | 0.32 (0.004) | | |
| Soil exch / compost total Mg (% DM) | 0.011 (0.0001) | | 0.49 (0.012) | | |
| Soil exch / compost total Ca (% DM) | 0.97 (0.007) | | 12.1 (0.12) | | |
| Soil exch / compost total B (mg.kg ⁻¹ DM) | 2 (0.03) | | 33.1 (0.13) | | |
| Copper (mg.kg ⁻¹ DM) | 19.8 (0.14) | 100 | 144.1 (0.84) | 1000 | 221 |
| Zinc (mg.kg ⁻¹ DM) | 78.2 (0.24) | 300 | 265.0 (5.49) | 3000 | 103 |
| Cadmium (mg.kg ⁻¹ DM) | 0.31 (0.002) | 2 | 0.8 (0.0) | 15 | 78 |
| Chrome (mg.kg ⁻¹ DM) | 67.3 (0.33) | 150 | 27.1 (0.65) | 1000 | 12 |
| Mercury (mg.kg ⁻¹ DM) | 0.06 (0.001) | 1 | 0.86 (0.06) | 10 | 435 |
| Nickel (mg.kg ⁻¹ DM) | 45.3 (0.17) | 50 | 16.5 (0.23) | 200 | 11 |
| Lead (mg.kg ⁻¹ DM) | 43.1 (0.26) | 100 | 57.3 (2.53) | 800 | 40 |

microorganisms, organic trace elements and trace metals. Soil and compost initial characteristics are shown in Table 1.

Field procedures

The N, P, cation and trace metal concentrations in the photosynthetic parts of four garrigue species were followed during two years after amendment. The control organs chosen for nutrition assessment are photosynthetic tissues because they constitute the centre of metabolism, and because their composition well reflects nutrition changes (Martin-Prével, Gagnard & Gautier 1984). *Quercus coccifera* and *Brachypodium retusum* were monitored because they are the two dominant species of the experimental site, reaching respectively nearly 40 and 70 % of cover. Two other species were chosen, *i.e.* *Cistus albidus* and *Ulex parviflorus*, in order to study several vegetation categories reactions to compost amendment for their nutrition. Indeed, we can segregate these species by life-forms, *i.e.* herb (*Brachypodium retusum*) and woody species (3 other species). Moreover, *Quercus coccifera* is an evergreen sclerophyllous shrub, while *Cistus albidus* is a semi-deciduous malacophyllous shrub. Finally, *Ulex parviflorus* distinguishes from the others as it is a Legume species with photosynthetic stems.

Four samples per compost rate were analyzed, each corresponding to one experimental plot. These analyzed samples were a mix of 3 samples randomly taken on each plot. Only leaves were collected for *Quercus coccifera* and *Cistus albidus*, while all green organs were collected for *Brachypodium retusum* and *Ulex parviflorus*. The plant parts were sampled 8 times from March 2002 to March 2004 (March, April, June, October 2002; March, June, October 2003; March 2004). The experiment begun in March 2002, one month and a half after amendment, as in Mediterranean region the vegetative growth starts at that period, and consequently compost effects on plant nutrition might just begin.

Laboratory procedures

The plant material was washed with demineralized water, oven dried at 45°C and crushed (1 mm) in a trace-metal-free grinder (made with tungstene carbure, FOSS TECATOR Sample Mill 1093 Cyclotec). For N, K, Ca and Mg analysis, 250 mg of sample were digested one night in 5 ml H₂SO₄ (96%) and 5 ml H₂O₂ (35%), then heated at 400°C during 3 hours (Bioblock Scientific Digestion Unit 10401). The solutions were diluted 500 times by adding

ultrapure water (Millipore Symplicity 185), filtered at 0.45 μ m and analysed by ion chromatography (Dionex DX120) (method adapted from [Masson and Andrieu 1996](#)). The eluent used was 26 mmol.L⁻¹ methan sulfonic acid. The efficiency was assessed by comparing the element concentrations obtained by the chosen method with values certified in plant reference material. Total plant N was slightly underestimated (86 % efficiency), while the cation concentrations were overestimated, especially for Ca (K: 105 %; Mg: 113 %; Ca: 125 % of efficiency). However, as the same method was used for all the samples, similar estimation error of concentrations occurred for each sample, and comparisons between rates and dates were possible.

Total concentrations of the other elements, P, B, Cd, Cr, Cu, Ni, Pb and Zn, were measured after digestion in *aqua regia* using atomic absorption spectrometry.

Statistical analyses

Two way ANOVA (species x rate; date x rate) combined with Tukey test ([Zar 1984](#)) were used to study compost rate effect on plant element concentrations. If any interaction occurred between the two studied factors, one way ANOVA were performed at each sampling date for each species to study compost rate effect. Significant level was considered to be 95 %. The softwares Statgraphics plus (version 2.1: Statistical Graphics Corporation,[®] Copyright 1994-1996) and Minitab (release 13 for Windows, 2000, Minitab Inc., USA) were used.

Results

Compost effect on N concentrations (Fig. 1)

Compost improved N nutrition of *Quercus coccifera*, *Brachypodium retusum* and *Cistus albidus* as early as April 2002 (two way ANOVA, species x rate, F=20.88, p<0.0001), but had no pronounced effect on *Ulex parviflorus*. N concentrations in green parts of the 3 first species were significantly higher overall on amended plots compared to control. The compost effect was proportional overall to the rate, as D50 N values were intermediate between control and D100. However, *B. retusum* was more sensitive to compost than the shrubs. N concentrations nearly doubled on D100 for *B. retusum* (June, October 2002 and March 2003), whereas it increased by 50 % for the two other species. However, *Cistus albidus* leaves had the highest concentrations in N, reaching a value over 2 % N on D100 in October 2003.

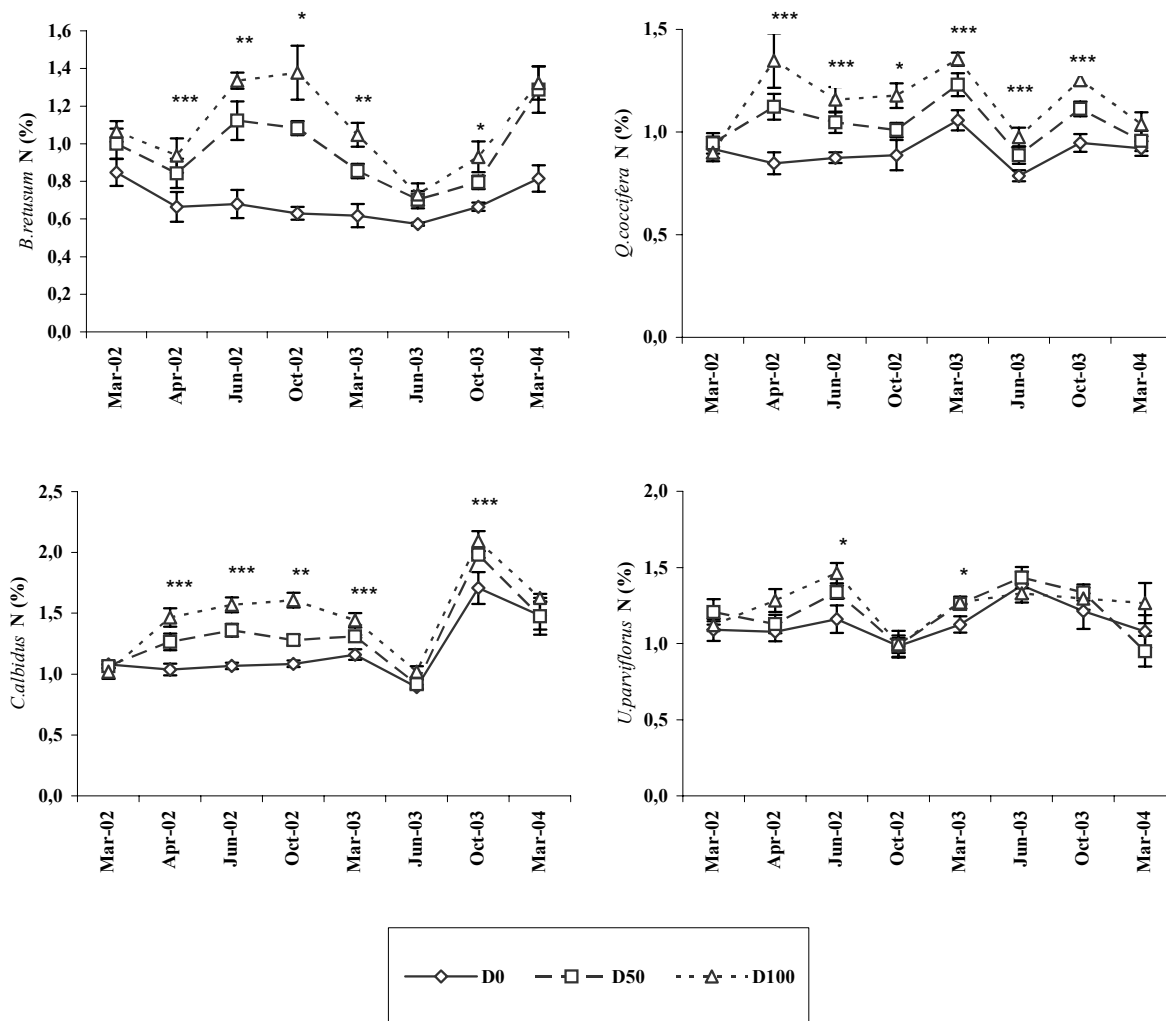


Figure 1. N concentrations in green parts of the 4 studied species, from March 2002 to March 2004, for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean \pm SE, N=4. *0.05<p<0.01; **0.01<p<0.001; ***p<0.001 (one way ANOVA).

Moreover, the lowest N concentrations were observed in June 2003 for the 3 species, and the compost improving effect disappeared at this date for *B. retusum* and *C. albidus*, whereas it remained significant for *Q. coccifera* (two way ANOVA, date x rate, $F=32.44$, $p<0.0001$). Finally, in March 2004, two years after amendment, the compost effect was still perceptible for *B. retusum* (one way ANOVA, $F=8.58$, $p=0.0082$), whereas it had disappeared for the 2 shrubs.

Compost effect on P concentrations (Fig. 2)

Compost at both rates significantly increased P concentrations in the green organs of the 4 species, but the patterns of change during the two years after amendment differed according to the species.

B. retusum was very sensitive to compost effects, as its P concentration increased significantly as early as March 2002 (one way ANOVA, $F=8.19$, $p=0.0193$), and as D100 concentrations were multiplied overall by a factor 3 compared to control.

Similarly, compost effects began in March 2002 for *C. albidus* (one way ANOVA, $F=47.02$, $p=0.0002$), but increased progressively during the experiment to reach a 3-times higher value on D100 compared to control, two years after amendment (March 2004) (one way ANOVA, $F=26.96$, $p=0.002$). As for N, *C. albidus* showed the highest P concentrations in leaves, reaching 0.4 % of P on D50 in March 2004.

Contrary to N, P concentrations in *U. parviflorus* were durably and significantly increased by compost, the concentrations on plots amended at both rates being twice as high as control. Compost effects on *U. parviflorus* began in June 2002 (one way ANOVA, $F=103.66$, $p<0.0001$) and persisted until March 2004 (one way ANOVA, $F=47.26$, $p<0.0001$).

Finally, compost had low effects on *Q. coccifera* P concentrations, which were higher on D100 (+ 50 %) compared to control in June and October 2002, and in October 2003 (two way ANOVA, date x rate, $F=15.15$, $p<0.0001$).

Compost effects on cation concentrations (Fig. 3)

Compost had improving effects on *B. retusum* cation nutrition as early as March 2002 for Mg and Ca (one way ANOVA, Mg: $F=4.16$, $p=0.049$; Ca: $F=5.78$, $p=0.0274$), in April 2002 for K (one way ANOVA, $F=21.72$, $p<0.0001$). The cation concentrations were significantly higher on amended plots compared to control, and these effects persisted until March 2004 (one way

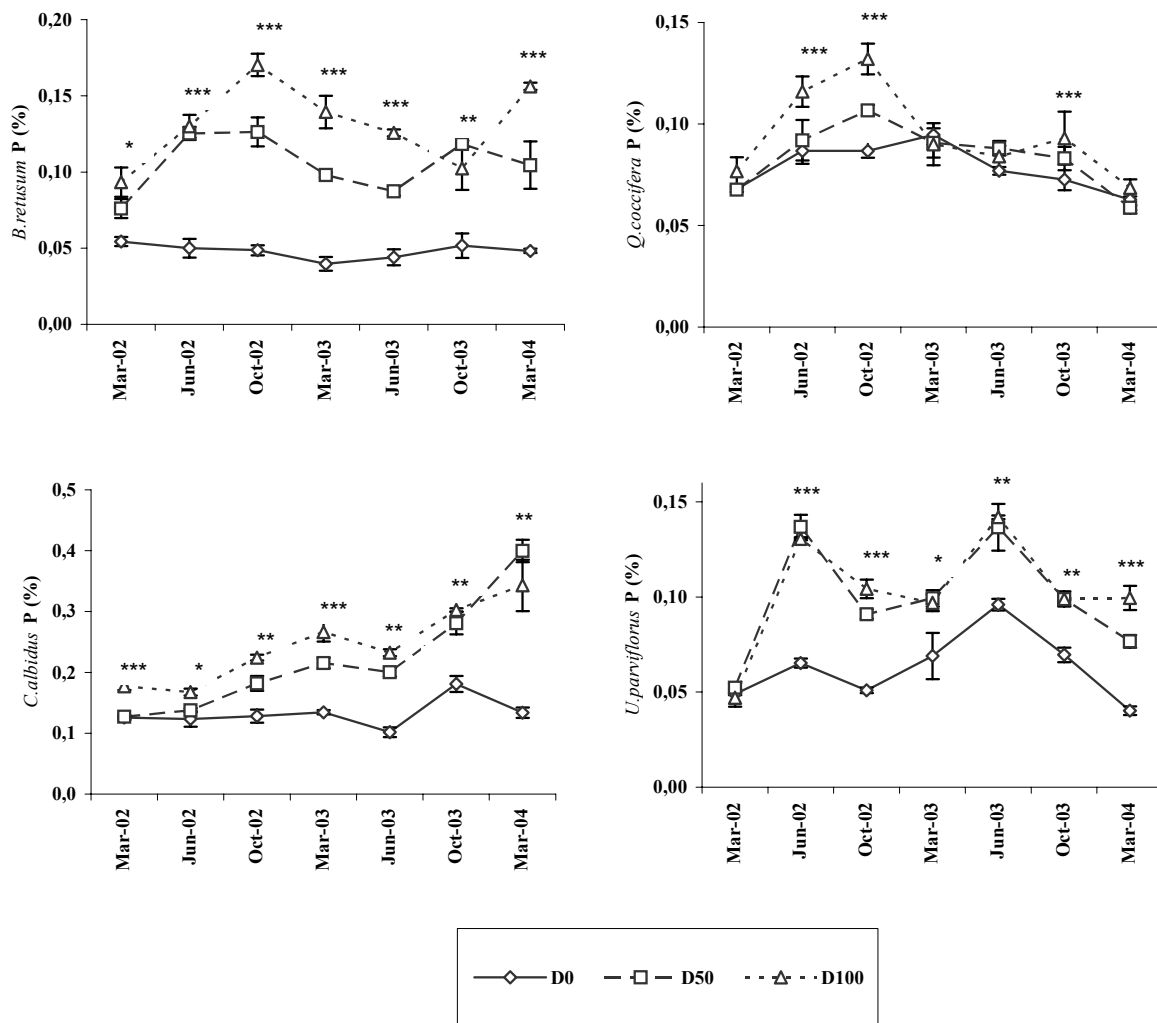


Figure 2. P concentrations in green parts of the 4 studied species, from March 2002 to March 2004, for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean \pm SE, N=4. *0.05<p<0.01; **0.01<p<0.001; ***p<0.001 (one way ANOVA).

ANOVA, K: $F=10.14$, $p=0.0049$; Mg: $F=9.02$, $p=0.0071$) (two way ANOVA, date x rate, Ca: $F=14.33$, $p<0.0001$). However, K concentrations decreased strongly on amended plots from one experiment year to another.

Compost improved *C. albidus* and *Q. coccifera* K concentrations as early as April 2002 (two way ANOVA, species x rate, $F=21.72$, $p<0.0001$). *C. albidus* K concentrations remained significantly higher on amended plots until March 2004 (two way ANOVA, date x rate, $F=38.66$, $p<0.0001$), whereas they returned to control values at this date for *Q. coccifera*, after a decrease during the second year.

Compost had no effect on *Q. coccifera* Mg concentrations and a low effect on *C. albidus* Mg concentrations, the latter appearing sporadically significant in October 2002 (one way ANOVA, $F=4.61$, $p=0.0418$), June 2003 (one way ANOVA, $F=17.59$, $p=0.0008$), and March 2004 (one way ANOVA, $F=5.37$, $p=0.0291$).

Compost had antagonistic effects on *B. retusum* Ca concentrations and woody species Ca concentrations. Compost significantly increased Ca concentrations in green parts of *B. retusum*, whereas it significantly decreased them for *Q. coccifera* and *C. albidus*. However, this effect had disappeared for the two woody species two years after amendment. *C. albidus* showed the highest concentrations in Mg and Ca, while *U. parviflorus* had the highest K concentrations.

Finally, *U. parviflorus* was not sensitive to compost for its K, Mg and Ca nutrition. However, this species showed a marked seasonality for its cation nutrition, with peaks of absorption in spring (April and June) for both experimental years.

Compost effect on trace metal concentrations

Compost had a low effect overall on the trace metal concentrations of green parts.

The most sensitive species was *C. albidus*, whose Cd and Pb concentrations were sporadically affected by compost presence. For this species, Cd concentrations were significantly lower on amended plots compared to control, for both March 2003 (D100: 0.2 ± 0.01 , D50: 0.2 ± 0.02 , and D0: 0.4 ± 0.04 mg.kg⁻¹) (one way ANOVA, $F=18.93$, $p=0.0006$) and March 2004 (D100: 0.11 ± 0.02 , D50: 0.21 ± 0.03 , and D0: 0.21 ± 0.001 mg.kg⁻¹) (one way ANOVA, $F=11.18$, $p=0.0036$). In contrast, in March 2002, *C. albidus* Pb concentrations were significantly increased on D100 compared to control (D100: 3.8 ± 0.6 , D50: 2.3 ± 0.1 , and D0: 1.7 ± 0.2 mg.kg⁻¹) (one way ANOVA, $F=8.72$, $p=0.0078$).

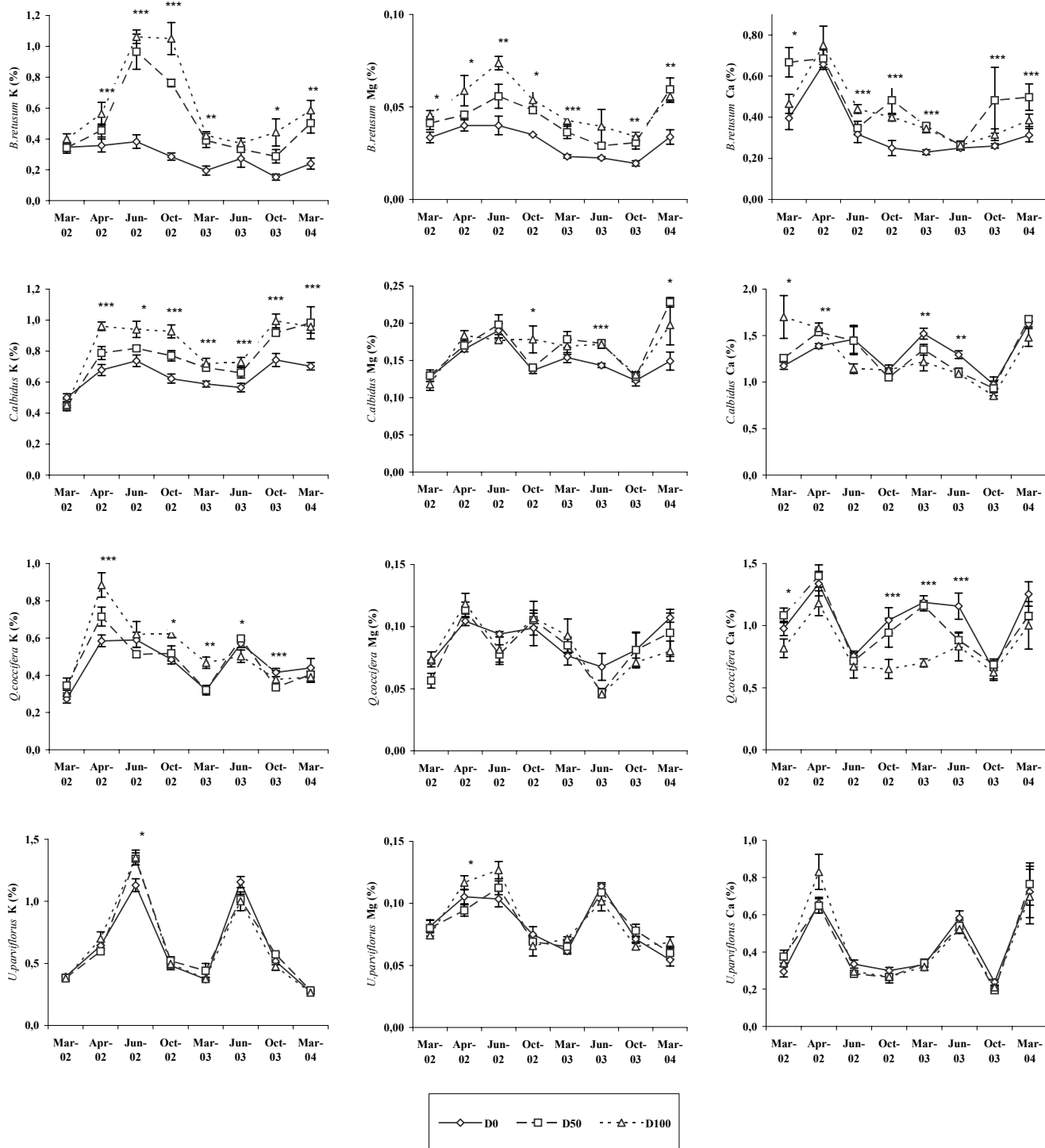


Figure 3. K, Mg and Ca concentrations in green parts of the 4 studied species, from March 2002 to March 2004, for D0 (control), D50 (50 Mg.ha⁻¹ of compost), and D100 (100 Mg.ha⁻¹ of compost) plots. Mean \pm SE, N=4. *0.05<p<0.01; **0.01<p<0.001; ***p<0.001 (one way ANOVA).

Moreover, *Q. coccifera* and *B. retusum* showed opposite effects of compost on B concentrations in March 2003. B concentrations were significantly higher on D100 (5 ± 0.2 mg.kg⁻¹) for *B. retusum* compared to control (3.7 ± 0.3 mg.kg⁻¹) (one way ANOVA, $F=5.83$, $p=0.0238$), whereas they were significantly lower on the same plots for *Q. coccifera* (D100: 36 ± 2.3 ; D0: 21 ± 3.2 mg.kg⁻¹) (one way ANOVA, $F=8.98$, $p=0.009$).

Discussion

Overall, compost increases the macronutrient concentrations of plant photosynthetic organs, especially N and P, as compost greatly enriches the soil in these elements, and thus improves their availability for root absorption. This shows that even if adapted to low-resource ecosystem, garrigue plants species are reactive to fertilization. This is consistent with the results of other studies of compost effects on Mediterranean vegetation. [Moreno et al. \(1996\)](#), as well as [Martinez et al. \(2003a\)](#), have reported that plants grown on compost amended soils showed higher N, P and K concentrations than control plants. Moreover, in our study, the compost effect is proportional overall to application rate, as noted by [Martinez et al. \(2003a\)](#). The five macronutrient concentrations in plants were increased, which indicates that no immobilization or antagonism occurred between elements supplied by compost to the soil. [Ballard \(1984\)](#) notes that application of P in conjunction with N and K often has a synergistic effect on the utilization of these elements.

The compost effects on vegetation nutrition began as early as March or April 2002, depending on the species considered. The optimal season for growth under Mediterranean climatic conditions is spring, when neither water availability, nor low temperature are limiting ([Larcher 1983](#); [Orshan 1989](#)).

Compost effect on the different macronutrients

The compost effect on plant nutrition was at a maximum for P, as all studied species increased their P content after amendment, and as the amplitude of these increases was the highest among all nutrients. The compost used is very rich in phosphorus, compared to N and K concentrations. This is due to the nature of the sewage sludge that enters the compost composition. This sewage sludge comes from a sewage plant that applies a dephosphatation process to the sewage. Thus P is concentrated in the sludge, and the latter greatly enriches the compost in P. Moreover, the compost P richness induced higher durability of P fertilization

effects, as P concentrations in all studied species remained higher on amended plots (except *Q. coccifera*) two years after amendment. However, compost N and cation improving effects also persisted at the end of the experiment for both *C. albidus* and *B. retusum*, implying that compost still provides N and cations. These results differ from several authors who showed the durability of compost fertilization effects to be limited (Guerrero *et al.* 2001; Martinez *et al.* 2003a). The compost used in our experiment is aged for seven months and is consequently relatively mature. Thus, the humified and stable nature of its organic matter may have induced a slow mineralization and release of macronutrients to soil and vegetation.

Compost effect on Mg concentration is very low for the three woody species, whereas it is marked for the herb species, *i.e.* *B. retusum*. Herb species are known to be more reactive to fertilization than woody species (Berendse 1998).

Compost had opposite effects on bushes and herb Ca concentrations. Compost increased this element concentration in *B. retusum*, while its concentrations decreased in *Q. coccifera* and *C. albidus* leaves. The decrease in the two shrubs should be linked with a nutrition stress decrease, as compost greatly enriches the soil in plant nutrients. Ca plays a role in the construction of the plant pecto-cellulosic wall, and is involved in the sclerosis process (Martin-Prével 1978), which increases with plant stress level. For *B. retusum*, as this element is necessary to the cell elasticity in the elongation process (Martin-Prével 1978), Ca could be involved in the increased growth rate that is frequently observed after improvement of N nutrition (Glass 1988).

Compost effect on plant trace element contamination

Compost had no significant effect overall on trace element concentrations in plants. Thus, although the compost probably increased concentrations of these elements in the soil, this did not increase their absorption by plants. Similarly, Martinez *et al.* (2003a) reported that Pb, Ni, Cd and Cr concentrations in plant tissue did not increase significantly after compost amendment. Trace metals in the soil solution may be reduced to a non-toxic level through complexation with soil organic substances, when the metal-organic complex has low solubility, as in the case of complexes with humic acids and other high-molecular-weight components of organic matter (Kim, Power & Bell 2003). Ni, and particularly Cu, are known to be retained by organic matter and in this way are not transferred to plants (Moreno *et al.* 1996). Moreover, Ca, P, and Mg are the main antagonistic elements against the plant

absorption and metabolism of several trace elements, such as Cd, Pb and Ni (Kabata-Pendias & Pendias 1992). Finally, in calcareous soils, the high pH favours the immobilization of most trace metals (Moreno *et al.* 1996; Martinez *et al.* 2003a).

However, some slight compost effects sporadically appeared on plant trace element concentrations. In March 2003, compost increased B concentration in *B. retusum*, whereas it decreased B concentration in *Q. coccifera* leaves. This might be related to the increase of Ca concentration in *B. retusum* as opposed to the decrease of Ca concentration in *Q. coccifera* induced by compost. This element has antagonistic effects on B availability for many plants (Chapman 1966; Kabata-Pendias & Pendias 1992).

It seems that the organic matter brought by compost to soil has decreased *Cistus albidus* Cd absorption in March 2003 and 2004. Kim *et al.* (2003) reported lower Cd levels in shoots of *Pinus sylvestris* seedlings grown in organic soil compared to those grown on mineral soil.

Pb concentration in *C. albidus* leaves doubled just after amendment, but the new concentration persisted in the normal concentration range reported in plants by Kabata-Pendias & Pendias (1992). The decrease of Ca concentration in leaves of this species might be linked with the increase of their Pb concentration, as Ca is antagonistic to Pb, and as Pb can mimic the physiological behavior of Ca in plants (Kabata-Pendias & Pendias 1992).

Plant species' reactions to compost amendment

Species' nutrition responses to compost amendment vary greatly according to their life-traits. *B. retusum* is the most sensitive species to compost fertilization effects for its nutrition. First, it is the most precocious, as its response to compost amendment begins as soon as March 2002 for P, Mg, and Ca. Second, its response to compost is of greater amplitude compared to shrub species. Similarly, Berendse (1998) reports that grass species respond much more rapidly than shrub species to an increased nutrient supply. Moreover, *B. retusum* roots colonize the 5-10 upper cm of the soil (Caturla *et al.* 2000) and can probably directly dip into compost reserves.

In contrast to *B. retusum*, *U. parviflorus* is the least sensitive species to compost amendment for its nutrition. P is the only element whose concentration increases with compost amendment. As this species can fix atmospheric N₂ by means of its Rhizobium symbiotes (Quispel 1983), compost has a poor effect on its N nutrition. Moreover, this species shows a clear seasonal pattern for macronutrient absorption, with higher values reached in spring (Ballini 1993).

C. albidus is the most reactive shrub species to compost amendment. Likewise, [Guerrero et al. \(2001\)](#) reported that compost application increased this species' N, P, K and Mg content. *C. albidus* develops on low depth soils and as a semi-deciduous shrub may have a superficial root system ([Margaris 1981](#)). Thus, the roots of this species should have colonized the soil zone beneath compost mulch and consequently might greatly benefit from the elements leached from the soil surface. This species shows the highest leaf N, P, Mg and Ca concentrations compared to the 3 other species studied, according to the reported nutrient accumulation strategy of *Cistus* species ([Carreira & Niell 1992](#)).

Finally, *Q. coccifera* shows intermediate behaviour with regard to compost nutrition improvement. For this species, N, P, and K concentrations are increased by compost amendment. [Rapp et al. \(1999\)](#) report increased availability of N accelerated uptake and accumulation in tissues of Mediterranean oaks. However, compost fertilization effects on *Q. coccifera* decreased over time for N, and especially P and K, probably due to the progressive decrease of nutrient pools in compost. Nutrients become less available for deep roots of *Q. coccifera*.

The year 2003 has been particularly dry in Southern France, and the drought effects began as early as spring. *Q. coccifera* showed the best resistance capacity to drought. In June 2003, the N concentration of the species studied (except *U. parviflorus*) decreased to reach minimum values over the two years of experiment. Over a period of water stress, a marked decrease in nutrient uptake can occur. This is due to a decreased transfer of ions to the roots, a possible reduction in root absorption ability, associated with reduced plant assimilation (caused by moisture stress), and to morphological factors, such as more rapid suberization of roots with water stress ([Bowen 1984](#)). At this date, the compost improving effect on N concentration disappeared sporadically for *B. retusum* and *C. albidus*, whereas it persisted for *Q. coccifera*. This species' deep extensive root system might have protected it from drought effects, as it allows access to deep underground soil moisture reserves ([Keeley 1986](#)). Moreover, as fertilizers often promote root growth, particularly in the enriched area ([Bowen 1984](#)), the compost mulch could have induced root development of *C. albidus* and *B. retusum* at the soil surface, or even in the compost mulch. As these zones are primarily prone to desiccation under drought conditions, these species might have suffered more from drought on amended plots compared to deep rooting *Q. coccifera*.

Compost effects on nutrient cycling in soil / plant system

The increase of nutrient concentrations in soil and plants due to compost amendment should lead to an increase in litter nutrient content. The nutrient content in litter is related to soil nutrient availability, uptake and translocation to young tissues (Gosz 1984). Rapp (1999) showed that translocation of elements before leaf fall decreases with the nutrient availability of the site. In addition, the plant nutrient enrichment induced by compost should increase litter decomposability, especially the improvement of plant N status. Increasing N foliar content decreases the leaf polyphenol and organic acid levels by decreasing the plant stress level, and consequently increases litter decomposition and mineralization rates (Gosz 1984; Miller 1984). Thus, the plant nutrition improvement induced by compost can accelerate the nutrient flux through the soil-plant system by first improving litter quality, and secondly by increasing litter decomposability, which enhances soil nutrient availability for plants.

However, the four species studied may not play an equal role in this nutrient cycle improvement. *B. retusum* and *C. albidus* may be the two major improving species, as they are the most reactive species to compost amendment and greatly increase their nutrient contents. In addition, both species might have high litter production rates and high litter decomposability rates. As a herb, the leaves of *B. retusum* die every year and provide fresh litter. Similarly, *C. albidus* leaves are shed when no older than one year (Floret *et al.* 1989). Thus, these two species reconstitute their entire foliar biomass to soil every year. Furthermore, both species have poorly lignified leaves and a significant inverse correlation has been assessed between litter initial lignin percentage and its decomposition rate (Gosz 1984; Berendse 1998).

In contrast, *Quercus coccifera* has delayed nutrient restitution to soil, as only one third of its leaf biomass is shed every year. Thus, even though its nutrient status is improved by compost, it plays a lesser role in the nutrient cycle improvement. In addition, this species sclerophyllous leaves contain high concentrations of phenolics and lignins, which are not only resistant to decomposition, but may also bind other elements into resistant organic complexes (Archibold 1995).

U. parviflorus greatly benefits from compost effects for its P nutrition, and may play a role in P cycle improvement in the ecosystem. However, this species' litter decomposes slowly (Ballini & Bonin 1995), as it is greatly lignified. Thus, this species' enhancement effect on P cycling may also be delayed compared to *B. retusum* and *C. albidus*.

Plant reactions can be separated into two categories according to the time-scale at which they should improve nutrient turnover. Species with superficial rooting and short life duration of leaf, *i.e.* *Cistus albidus* and *Brachypodium retusum*, rapidly increase nutrient turnover, while species with deeper rooting and sclerophyllous leaves, *i.e.* *Ulex parviflorus* and *Quercus coccifera*, have more delayed improving effects.

Moreover, three nutrition strategies occur among garrigue bushes. The semi-deciduous *Cistus albidus* has a nutrient accumulation strategy, while sclerophyllous and evergreen *Quercus coccifera* appears stress resistant and the Legume *Ulex parviflorus* opportunistic. Similarly, Kruger (1987), by analysing the responses of Mediterranean plants to seasonal availability of minerals, suggested that there is great diversity of nutrition among the species within infertile habitats, according to plant uptake capacity.

All areas subject to the Mediterranean type of climate show indisputable similarities in the general aspect of their vegetation (Quézel 1981): evergreen trees and shrubs are the predominant life-forms present, and drought deciduous shrubs are also important, particularly in California and Chile (Mooney 1981). In addition, Legume species are widespread in the Mediterranean climate, both as woody shrubs of the mature stages and as short-lived, pioneer herbaceous taxa (Arianoutsou & Thanos 1994). An increase in nutrient resources of these ecosystems, by stimulating complementary resource use, should increase productivity because some species could aid the growth of others, either through provision of resources, or improvement of harsh environmental conditions (Hooper 1998). This shows that such communities are rather adapted to recurrent perturbation than to low resource environments, and that nutrient impoverishment after fire is one of the key factors limiting the succession processes in Mediterranean areas.

Aknowledgements

This research was commissioned by the Conseil Général des Bouches-du-Rhône (France), and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the French Rhône-Méditerranée-Corse Water Agency.

Résultats

Dès mars ou avril 2002, l'apport de compost a globalement augmenté les teneurs en macronutriments (N, P, K, Mg, Ca) des organes photosynthétiques des plantes étudiées, ce qui indique qu'aucun phénomène d'immobilisation dans le sol ou d'antagonisme vis-à-vis de l'absorption par les plantes ne s'est produit. L'effet fertilisant du compost est maximal et durable (2 ans) pour le P. Pour N et K, la durabilité est légèrement moindre que pour P car limitée aux espèces les plus sensibles à l'amendement pour leur nutrition. Aucune contamination sérieuse des plantes en éléments trace métalliques n'a pu être mise en évidence après amendement.

Les réponses des espèces à l'amendement varient fortement en fonction de leurs traits de vie. *B. retusum* est l'espèce la plus sensible à la fertilisation, son enracinement superficiel lui permettant de puiser directement les réserves de nutriments à partir de la couche de compost déposée sur le sol. A l'opposé, *U. parviflorus* est l'espèce la moins sensible à l'amendement car elle ne réagit qu'à l'apport de P, étant capable de fixer le N₂ atmosphérique. Au sein des arbustes, *C. albidus* est le plus réactif à l'amendement pour sa nutrition, en partie grâce à son enracinement superficiel. Enfin, *Q. coccifera* développe un comportement intermédiaire car les effets fertilisants du compost sur sa nutrition s'estompent la deuxième année, probablement du fait de son enracinement profond et de la diminution de la réserve en nutriments du compost. Par contre, cette espèce conserve des teneurs foliaires en N supérieures sur parcelles amendées par rapport au témoin, même en conditions drastiques de sécheresse. *Q. coccifera* montre donc la meilleure capacité de résistance à la sécheresse vis à vis de sa nutrition azotée.

L'amélioration de la nutrition des plantes de la garrigue après amendement pourrait accélérer les flux de nutriments au sein du système sol / plante, en augmentant la décomposabilité de la litière produite (augmentation de la teneur en N). *B. retusum* et *C. albidus* sont vraisemblablement les deux espèces à effet prépondérant sur cette accélération des flux de nutriments car ce sont les deux espèces les plus réactives à l'apport de compost. De plus, ces deux espèces ont une production de litière quantitativement importante et facilement décomposable. A l'opposé, *Q. coccifera* et *U. parviflorus*, qui produisent une litière lignifiée peu décomposable, ont un effet retardé sur les flux de nutriments.

Ainsi, cette étude met en évidence trois stratégies au sein des arbustes de la garrigue. Le ciste semi-décidu a une stratégie d'accumulation des nutriments, alors que le chêne sclérophylle et sempervirent apparaît résistant au stress, et l'ajonc (Fabacées) opportuniste. De plus, les

espèces à enracinement superficiel et courte durée de vie des feuilles (*B. retusum* et *C. albidus*) ont un effet immédiat sur le recyclage des éléments, alors que les espèces sclérophylles et à enracinement profond (*U. parviflorus* et *Q. coccifera*) ont un effet retardé.

Chapitre 3

DYNAMIQUE DE LA VEGETATION APRES AMENDEMENT DANS UN ECOSYSTEME MEDITERRANEEN POST-INCENDIE

Cet article présente l'évolution du recouvrement des principales espèces de la garrigue regroupées en fonction de leurs traits fonctionnels pendant les deux années après épandage.

Les conséquences de l'amendement ont été abordées séparément :

- l'écrasement de la végétation par le tracteur au moment de l'épandage,
- l'effet fertilisant du compost,
- le recouvrement du sol par une couche de compost parfois épaisse de dix centimètres.

Nos objectifs étaient de :

- Préciser les réactions de la végétation à l'apport de compost (recouvrement, traits de vie et stratégies de régénération, relations de compétition).
- Caractériser la part respective des trois effets de l'amendement précédemment cités dans la réponse de la végétation.

Vegetation dynamics after compost amendment in a Mediterranean post-fire ecosystem.

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Article soumis le 21 octobre 2004 à Agriculture, Ecosystems & Environment

Abstract

On Mediterranean calcareous soils, high fire frequency induces soil impoverishment and the development of stable *Quercus coccifera* garrigues, which delay the establishment of natural tree species. Organic amendment could increase soil fertility levels, alter the vegetation dynamics and the established dominance relationships in these communities, and could improve succession processes. In this study, the plant cover evolution was monitored in a burnt shrub amended ecosystem during two years. Three treatments were studied: control, 50 Mg.ha⁻¹ and 100 Mg.ha⁻¹ of fresh co-composted sewage sludge and green waste.

Amendment had two distinct effects on vegetation cover. First, the spreading process led to the crushing of the vegetation, which constitutes a disturbance. On D100, the squashing intensity reached a threshold, which limited the woody species' recovery after amendment. Thus, on these plots, *B. retusum*, as the dominant herb, was favoured, especially at the end of the experiment (two years after amendment). On D50, in contrast to D100, woody species were favoured compared to herbs, probably due to the space colonization strategy of *Q. coccifera* after crushing. Thus, compost at both rates favoured the two dominant resprouter species, *i.e.* *B. retusum* and *Q. coccifera*. However, resprouter cover increase was limited in time to the first year, before they had colonized all the free space created by squashing.

Secondly, compost had some fertilizing effect on *R. officinalis* (N, P) and *U. parviflorus* (P), and these two species' cover was improved on D50. However, compost also increased *U. parviflorus*' sensitivity to drought.

At the end of the experiment, a third compost effect appeared, as seeder cover became greater than D0 on D50. After amendment, compost covered rocky stones, which created a new territory where species with superficial rooting can establish (mulch effect). Heliophilous seeder species, such as *R. officinalis* and both *Cistus* species, might be able to colonize this open space as compost might favour their newly-produced seed germination.

Keywords: sewage sludge compost, *Quercus coccifera* L., *Brachypodium retusum* Pers., *Ulex parviflorus* Pourr., *Rosmarinus officinalis* L., competition, cover, life-forms, resprouters, seeders.

Introduction

The Mediterranean climate is characterized by long dry summers, as well as frequent strong and dry winds that both favour the spread of forest fires (Scarascia-Mugnozza *et al.*, 2000; De Luis *et al.*, 2001). In Provence, the acceleration of the disturbances frequency, especially fire, due to anthropization and expanding human populations (Dell *et al.*, 1986), induced the colonization of the most degraded zones by *Quercus coccifera* garrigues (Barbero, 1990). In very degraded lands prone to recurrent fires, *Quercus coccifera* garrigues can be considered as a blocking stage of succession (Lepart and Escarre, 1983; Quézel and Médail, 2003), or even as a pseudo-climax (Cañellas and San Miguel, 1998). Indeed, this resprouting species has a quite long-lived and it forms relatively stable communities (Keeley, 1986). Moreover, after fire, *Quercus coccifera* rapidly occupy the open space by vegetative multiplication and consequently obstruct the other species installation (Lepart and Escarre, 1983). After a disturbance, Mediterranean ecosystems tend toward regeneration of the initial state by two reproductive strategies: resprouting (vegetative reproduction) and seeding (sexual reproduction) (Baeza *et al.*, 2003). Resprouting occurs by recruitment of new shoots from above- or under-ground organs after the removal or damage of above-ground parts (Lloret and Vilà, 1997). Seeder species regenerate by seedling recruitment from a dormant seed bank (Calvo *et al.*, 2000a). Vegetative growth, including resprouting after loss of biomass, concerns the maintenance of the current generation, while seeding concerns the production of future generations (Bellingham and Sparrow, 2000).

Moreover, fires eliminate plant cover and leave the soil unprotected against the impact of raindrops (De Luis *et al.*, 2001). As Mediterranean region is characterized by violent precipitation events (Scarascia-Mugnozza *et al.*, 2000), the continual removal of weathered

rock material has resulted in rather thin, infertile soils, which are often deficient in organic matter, nitrogen and phosphorus (Le Houerou, 1973). Thus, the dominant species that grow under Mediterranean climate are also frequently adapted to low resource availability. Indeed, in habitats in which resources are limited, naturally occurring species have traits that allow them to effectively function for long periods under stress (Tenhunen *et al.*, 1984). Furthermore, growth analyses have shown that nutrient poor habitats are dominated by species with a low potential growth rate, whereas nutrient rich habitats are dominated by species with a high potential growth rate (Aerts and Van Der Peijl, 1993). Similarly, Berendse (1998) has shown that an increase in nutrient disponibility leads to species with low maximum growth rates and low biomass loss rates being replaced by species with high potential growth rates and high biomass loss rates. This implies that competition between plant species and the resulting species composition of the plant community can be strongly affected by changes in nutrient supply (Tilman,1984). In addition, although disturbance is usually considered to be the primary determinant of resprouting in plant communities, there is also evidence that site productivity (*e.g.* moisture and / or fertility) is also a contributing factor to the trade-off between resprouting and seeding (Bellingham and Sparrow, 2000). Thus, a change in site fertility could affect vegetation composition relatively to plants' growth and litterfall, as well as relatively to their regeneration strategy after disturbance. Yet, could compost modify the dominance relationships between species adapted to recurrent disturbances ? Indeed, organic amendment can raise soil fertility by increasing water-holding capacity (Caravaca *et al.*, 2002) and organic matter content, as well as nutrient content (Brockway, 1983; Martinez *et al.*, 2003a).

Most authors have studied compost effects on natural vegetation recovery just after a disturbance had occurred, when the vegetation cover has been destructed, while we studied interspecific relationship changes in a seven-year-old garrigue. Compost amendment consequences were separated between fertilization effect, squashing effect of amendment process, and mulch effect, which led to different responses. Our aims were to (i) determine the vegetation reactions to compost during two years after amendment (plant cover, life-traits and regeneration strategies, competitive relations), and (ii) characterize the respective part of each amendment induced effect (fertilization, mulch, squashing) on the vegetation response.

Material and methods

Study site and experimental design

The experiment was carried out on 6000 m² in the plateau of Arbois (Southern Provence, France; 5°18'6''E – 43°29'10''N in WSG-84 Norm) at 240 m above sea level and under Mediterranean climatic conditions. The soil was a silty-clayey chalky rendzina, with a high percentage of stones (77 %) and a low average depth (24 cm). The last fire occurred in June 1995 and the site was colonized by typical Mediterranean sclerophyllous vegetation, which belongs to the hoalm oak (*Quercus ilex* L.) succession series.

Compost was surface applied in January 2002. It was put in a tipper-wagon pulled by a tractor, which went over each plot, step by step, on length lines (Photo 1). The experimental design was a complete randomized block of twelve plots of 500 m². Four plots did not receive any compost (D0=control), four received 50 Mg.ha⁻¹ (D50), and four 100 Mg.ha⁻¹ of fresh compost (D100). Inside each 500-m² plot, a subsoiling line was remaining from an ancient plantation done on the site in 1970. These 1-m wide subsoiling lines were length-crossing the plots. Their percentage of outcropping stones was very elevated and consequently, their vegetation cover was very low.

Compost

The compost was produced by a local company (Biotechna, Ensues, Southern Provence) and is certified conform to the French norm on composts made with materials of water treatment origin (NF U 44-095, 2002). This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in swathes. The swathes were mixed several times in the next 6 months to promote organic matter humification. The final compost met the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals. Soil and compost characteristics are shown in Table 1.

Table 1. Organo-mineral A horizon (0-24 cm: maximal depth; N=12) and compost (N=3) physico-chemical characteristics. DM: dry matter. FM: Fresh Mass.

| Parameter | Soil | | Compost | |
|----------------------------------|---------------|---|---------------|---|
| | Mean (SE) | Allowed French limit value before sewage sludge amendment | Mean (SE) | Allowed French limit value (08/01/1998) |
| pH _{H2O} | 7.34 (0.008) | | 7.7 (0.05) | |
| Humidity (% FM) | | | 4.8 (0.29) | |
| CEC (meq.100 g ⁻¹) | 23.12 (0.31) | | | |
| Total Calcareous (%DM) | 4.17 (0.13) | | | |
| OM (% DM) | 7.58 (0.12) | | 46.8 (2.74) | |
| total N (% DM) | 0.36 (0.005) | | 2.03 (0.03) | |
| C/N | 12.42 (0.09) | | 13.4 (0.78) | |
| total P (% DM) | 0.037 (0.001) | | 3.24 (0.03) | |
| Exchangeable P (ppm) | 23.3 (0.35) | | 2514.8 (7.82) | |
| Copper (mg.kg ⁻¹ DM) | 19.8 (0.14) | 100 | 144.1 (0.84) | 1000 |
| Zinc (mg.kg ⁻¹ DM) | 78.2 (0.24) | 300 | 265.0 (5.49) | 3000 |
| Cadmium (mg.kg ⁻¹ DM) | 0.31 (0.002) | 2 | 0.8 (0.0) | 15 |
| Chrome (mg.kg ⁻¹ DM) | 67.3 (0.33) | 150 | 27.1 (0.65) | 1000 |
| Mercury (mg.kg ⁻¹ DM) | 0.06 (0.001) | 1 | 0.86 (0.06) | 10 |
| Nickel (mg.kg ⁻¹ DM) | 45.3 (0.17) | 50 | 16.5 (0.23) | 200 |
| Lead (mg.kg ⁻¹ DM) | 43.1 (0.26) | 100 | 57.3 (2.53) | 800 |



Photo 1. Compost spreading

Field procedures

Plant cover was monitored three times a year (March, June and October) during the two years following the amendment, on each 500-m² plot (4 repetitions per compost rate).

A non destructive method was chosen, *i.e.* the point intercept method, which used the idea that an infinite number of points placed within a vegetation sward will hit the plant species proportionally to their cover (Jonasson, 1983). Practically, a sharp rod, approximating a point, was vertically shifted on a line crossing vegetation and the contact occurrence of each plant species with the rod was noted. In each plot of 50 x 10 m, we set one line of 5m90 (60 reading positions) perpendicularly to the 50 m axis of the plot (Figure 1). This line covered perpendicularly the subsoiling line in each plot centre. Two parameters were calculated to assess plant cover: Centesimal Frequency (CF) and Specific Contribution (SC), defined as follows (Floret, 1988):

CF = number of contact occurrence on one line for one species / total reading positions of the line

SC = number of contact occurrence on one line for one species / number of contact occurrence for all the species.

CF represented the cover of each species and SC gave information about the relative cover of each species, *i.e.* about its cover competitive abilities.

The data were separated in different categories in order to precise the vegetation reactions to compost amendment. First, the total cover of the vegetation was measured. Second, the cover of the six major species (representing 95 % of the vegetation cover) colonizing the experimental site was measured: *Quercus coccifera* L., *Brachypodium retusum* Pers., *Cistus salviaefolius* L., *Cistus albidus* L., *Ulex parviflorus* Pourr., *Rosmarinus officinalis* L. Third, the species were segregated by life forms (woody species and herbs), and by their type of regeneration mechanism (resprouters and seeders).

Statistical analyses

Both one and two way ANOVA combined with Tukey test (Zar, 1984) were used to make comparisons of the plant covers according to the studied factors (date and compost rate). Significant level was considered to be 95 %. The softwares Statgraphics[®] plus (version 2.1:



Figure 1. Cover estimation by the point intercept method on a 5m90 line crossing vegetation for D0 (D0), D50 (50 Mg.ha⁻¹) and D100 (100 Mg.ha⁻¹) rates of compost.

Table 2. Results of one and two way ANOVA on cover and SC. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (a < b). D0: D0 plots; D50: plots amended with 50 Mg.ha⁻¹ of compost and D100: plots amended with 100 Mg.ha⁻¹ of compost.

| parameter | factor | F | p | Tukey's test |
|--|--------|-------|---------|---|
| Global cover in March 2002 | rate | 4.17 | 0.050 | D0 ^b D50 ^{ab} D100 ^a |
| Global cover from June 2002 to March 2004 | rate | 7.3 | 0.002 | D0 ^a D50 ^b D100 ^{ab} |
| Woody species cover from March 2002 to March 2004 | rate | 22.33 | <0.0001 | D0 ^a D50 ^b D100 ^a |
| Resprouter species cover from March 2002 to March 2004 | rate | 4.5 | 0.015 | D0 ^a D50 ^b D100 ^b |
| Seeder species cover from March 2002 to March 2004 | rate | 30.59 | <0.0001 | D0 ^b D50 ^b D100 ^a |
| Seeder species cover in March 2004 | rate | 12.22 | 0.0027 | D0 ^b D50 ^b D100 ^a |
| Woody species SC from March 2002 to March 2004 | rate | 10.34 | <0.0001 | D0 ^{ab} D50 ^b D100 ^a |
| Resprouter species cover from March 2002 to March 2004 | rate | 25.99 | <0.0001 | D0 ^a D50 ^a D100 ^b |
| Q. coccifera cover from March 2002 to March 2004 | rate | 6.24 | 0.003 | D0 ^a D50 ^b D100 ^a |
| U. parviflorus cover from March 2002 to March 2004 | rate | 3.57 | 0.034 | D0 ^a D50 ^{ab} D100 ^b |
| B. retusum SC from March 2002 to March 2004 | rate | 8.05 | 0.001 | D0 ^a D50 ^b D100 ^a |
| B. retusum SC in October 2003 and March 2004 | rate | 6.98 | 0.0057 | D0 ^a D50 ^a D100 ^b |
| U. parviflorus SC from March 2002 to March 2004 | rate | 3.66 | 0.031 | D0 ^a D50 ^{ab} D100 ^b |
| R. officinalis SC from March 2002 to March 2004 | rate | 3.37 | 0.041 | D0 ^{ab} D50 ^b D100 ^a |

Statistical Graphics Corporation, Copyright 1994-1996) and Minitab[®] (release 13 for Windows, 2000, Minitab Inc., USA) were used.

Results

B. retusum and *Q. coccifera* were the two major species at the experimental site, with respectively 65 and 40 % cover. (Figure 2). The perennial herb *B. retusum* was the dominant species. Its SC was 45 %. *Q. coccifera* was the dominant woody species with a SC of 25 %. During the two years of the experiment, these two species' cover was stable. Likewise, the relative cover (SC) of the different species remained the same from March 2002 to March 2004.

The amendment process (January 2002) induced the crushing of vegetation due to the movement of the tractor and tipper-wagon over the plots. In March 2002, 30.7 ± 0.5 and 39 ± 3.9 % of the vegetation remained squashed on D50 and D100, respectively. Consequently, in March 2002, overall cover was significantly lower on D100 compared to D0, D50 cover being intermediate (Table 2, Figure 3). However, the crushing effect on the cover disappeared in June 2002 for both rates, as the overall cover on D50 and D100 were similar to D0. From this date and until the end of the experiment, D50 vegetation cover was significantly higher than on D0 (Table 2). D100 vegetation cover reached D50 values in March 2003 and remained higher than D0 from this date until the end of the experiment (Figure 3).

Amendment at D50 increased significantly woody species' cover by an overall mean of 20 % compared to D0 (Table 2, Figure 4a). This group's cover remained around 80 % on these plots during the two years of the experiment, except just after the amendment in March 2002, and in October 2003, for which covers was lower. After squashing, the woody species' cover in D100 was lower than on D0 (Figure 4a). Then the cover rapidly increased and no significant differences were noted between D100 and D0. A significant decrease occurred on D100 in October 2003 (Table 2).

In March 2002, similarly to overall vegetation cover, resprouter cover (Figure 4b) and seeder cover (Figure 4c) were lower on amended plots compared to D0, D100 cover being lower than D50. Amendment at both rates significantly increased resprouter species cover (*Q.*

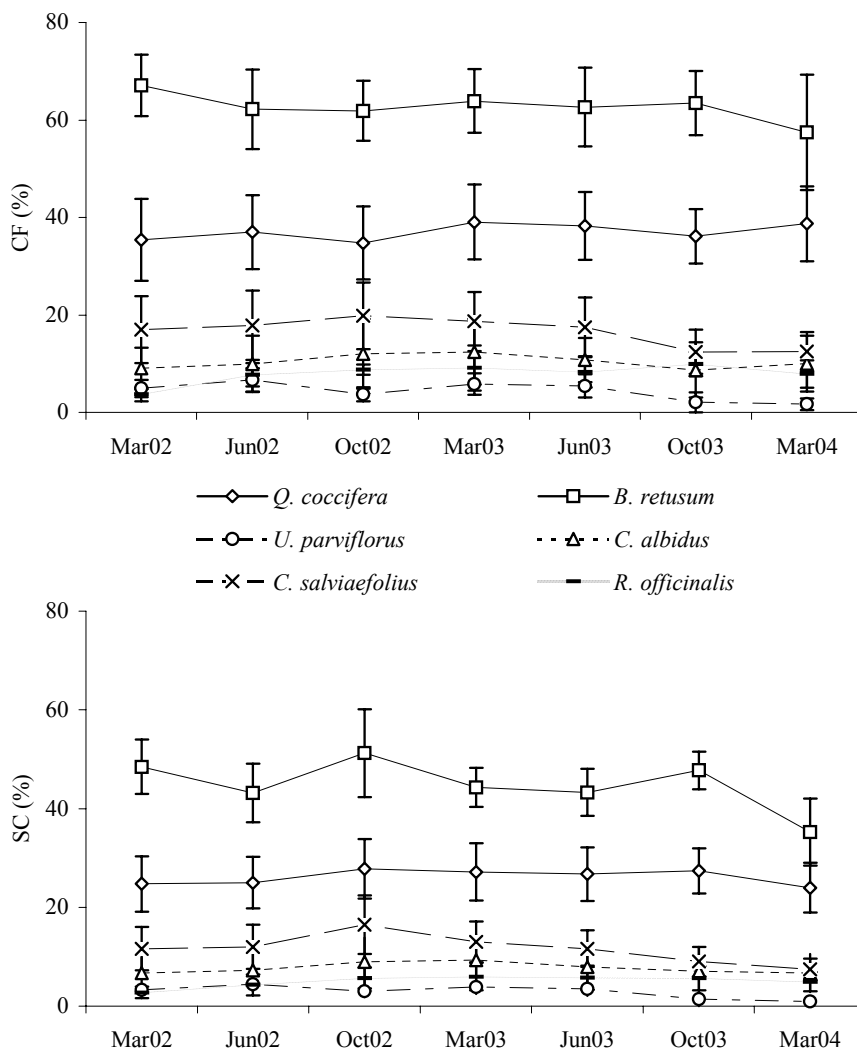


Figure 2. Species CF and SC evolutions on D0 plots during the two years of experiment. Mean \pm SE (N=4).

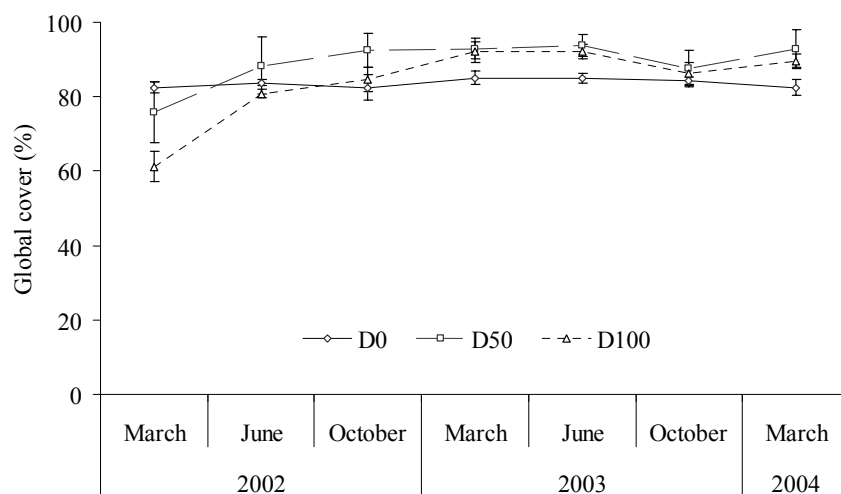


Figure 3. Global vegetation cover evolution during the two years after amendment for D0 (D0), D50 (50 Mg.ha⁻¹) and D100 (100 Mg.ha⁻¹) rates of fresh compost. Mean \pm SE (N=4).

coccifera and *B. retusum*) (Table 2). This compost effect occurred between June and October 2003, and persisted until March 2004, two years after amendment. In June 2003, seeder cover was similar for the three treatments. Then, from October 2003, it became higher than control on D50, while it was lower on D100.

The relative cover (SC) of resprouters (Figure 4e) significantly increased on D100 compared to D50 and D0 (Table 2). In October 2003, resprouters SC on both D100 and D0 plots strongly increased, D100 reaching 90 %.

Amendment at D50 had a significant stimulating effect on *Q. coccifera* cover (Table 2) compared to the other treatments (Figure 5a): this species reached a threshold (+ 20 %) in October 2002 until June 2003. At this date, *Q. coccifera* cover reached 60 % on D50. Then, it decreased to 50 % on D50 in October 2003 and March 2004, which was still higher than D0 and D100. No significant differences were noted between D100 and D0.

Amendment at D100 significantly increased *Ulex parviflorus* cover (Table 2) compared to D0 (Figure 5b). The cover on D100 remained highest from June 2002 to June 2003, then the cover decreased to D0 values. *U. parviflorus* cover on D50 followed the same pattern of change as on D100, but the values were intermediate between D0 and D100.

Compost at D50 significantly (Table 2) decreased *B. retusum* SC compared to D0 (Figure 5c). *B. retusum* SC was low from March 2002 to June 2003, then increased to D0 values in October 2003 and March 2004. *B. retusum* SC on D100 was below D0 values until June 2003 and became higher than other treatments from October 2003 to March 2004 ($p=0.006$).

On D100, *U. parviflorus* SC significantly increased compared to D0 (Table 2), but this effect disappeared in October 2003 (Figure 5d).

The two compost rates had contrasting significant effects on *R. officinalis* SC (Table 2, Figure 5e). Its SC decreased on D100, while it increased slightly on D50 compared to D0. Moreover, these effects remained until the end of the experiment.

Discussion

The repeated passage of the tractor on amended plots crushed the vegetation and induced a decrease in its cover, especially on D100 where the tractor went past twice to spread at double compost rate. Herb morphology implies a higher flexibility compared to woody species that constitutes an advantage with regard to the deleterious effects of crushing. Consequently, the

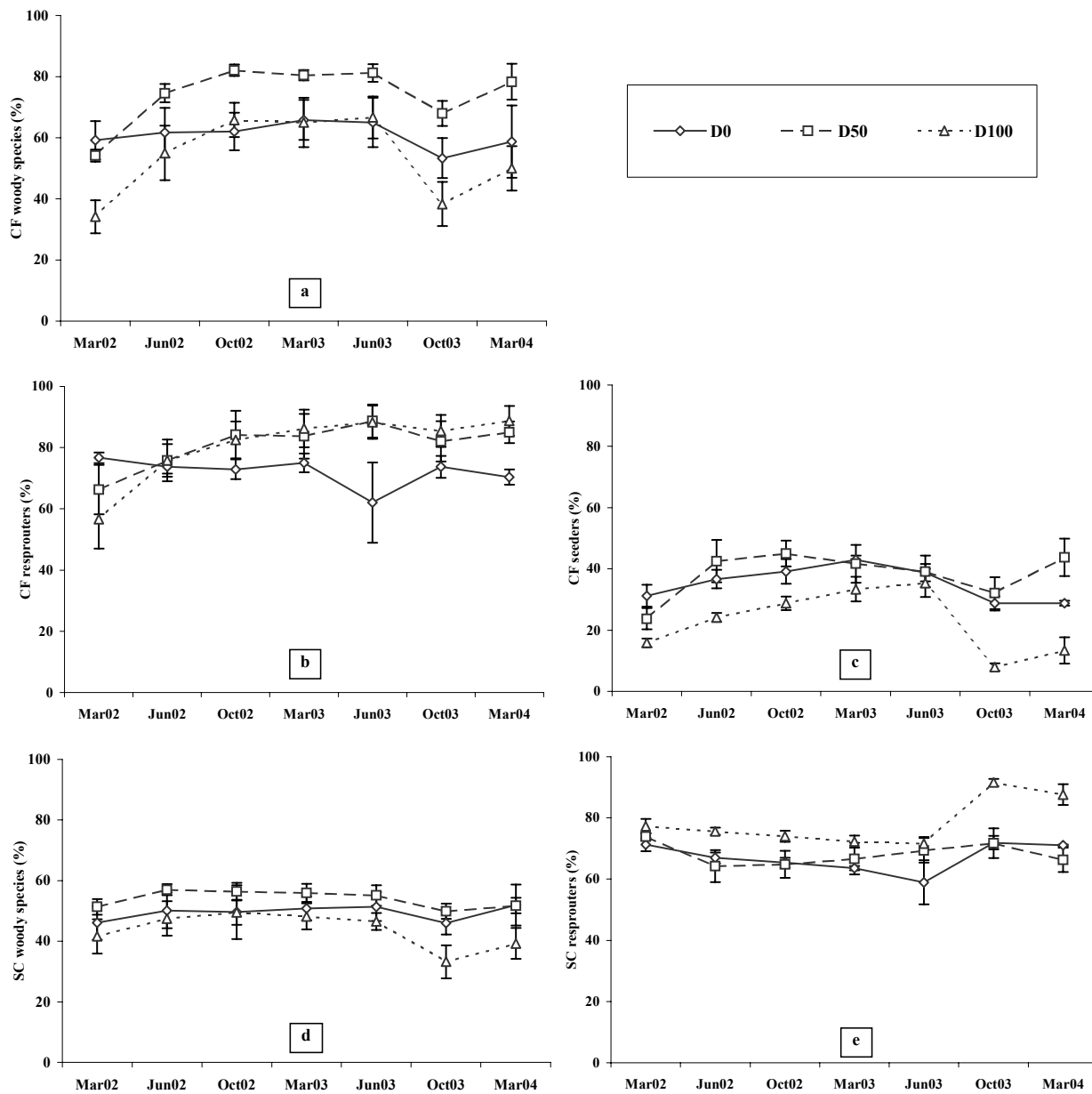


Figure 4. Life-forms and resprouter / seeder cover and SC evolutions during the two years after amendment for D0 (D0), D50 (50 Mg.ha⁻¹) and D100 (100 Mg.ha⁻¹) rates of fresh compost. Mean ±SE (N=4).

woody species SC decreased on D100 while it increased for herbs. No other study with compost spreading had revealed crushing effects on vegetation. Most studies deal with compost spreading in plantations or in burned lands, where the vegetation cover at amendment time is scarce. In contrast, the vegetation colonizing our experimental site was 7 years old at amendment time (last fire in 1995), and had a total cover of 80 %, with a 60 % bush cover. Thus, it was nearly impossible to spread compost manually, and we chose the mechanical amendment process used in agriculture. Consequently, in our study, the eventual fertilizing effects of compost will have to be separated from the impact of crushing.

From June 2002 to the end of the experiment, the amendment improved vegetation cover. Likewise, several authors have shown the improving effects on vegetation development (Moreno *et al.*, 1996; Navas *et al.*, 1999; Guerrero *et al.*, 2001; Martinez *et al.*, 2003a). However, due to the stronger crushing effects on D100 than on D50 at amendment time, the vegetation cover increase was not proportional to the compost rate in our experiment, contrary to the results obtained by Navas *et al.* (1999). D50 vegetation cover remained all the time higher than on D100.

From June 2002 for D50, and March 2003 for D100, overall vegetation cover reached higher values than D0. This shows the strong recolonisation capacity of the site vegetation after disturbance. The major plant and animal communities of Mediterranean regions have an exceptional capacity for recovery after disturbance, probably due to the severe environmental conditions imposed by the Mediterranean-type climate (Dell *et al.*, 1986). The recolonization of space by woody plants after crushing implies the development of young individuals, which can be sprouts in the case of *Q. coccifera*, or seedlings in the case of seeders. Resprouter species' response to fire is regulated by a positive feedback mechanism of vegetative regeneration, allowing quick space occupation often before seeding species have germinated (Caturla *et al.*, 2000; Calvo *et al.*, 2002b; Montès *et al.*, 2004). Consequently, the strong crushing effect of amendment induced an increase of resprouter species cover and domination, especially on D100. However, after an increase over 7 months from March 2002 to October 2002, this group's cover stabilized at around 85 % until the end of the experiment. This strong increase, followed by a halt in cover progression, suggests that from October 2002, all the open space created by squashing had been colonized. On the other hand, seeders were very affected by crushing, which induced a decrease in their cover on D100 throughout the experiment. For example, *R. officinalis* cover was significantly decreased on D100. However, on D50, from June 2002 to October 2003, seeder cover did not differ from D0, indicating that these species had recovered from the destructive effects of crushing.

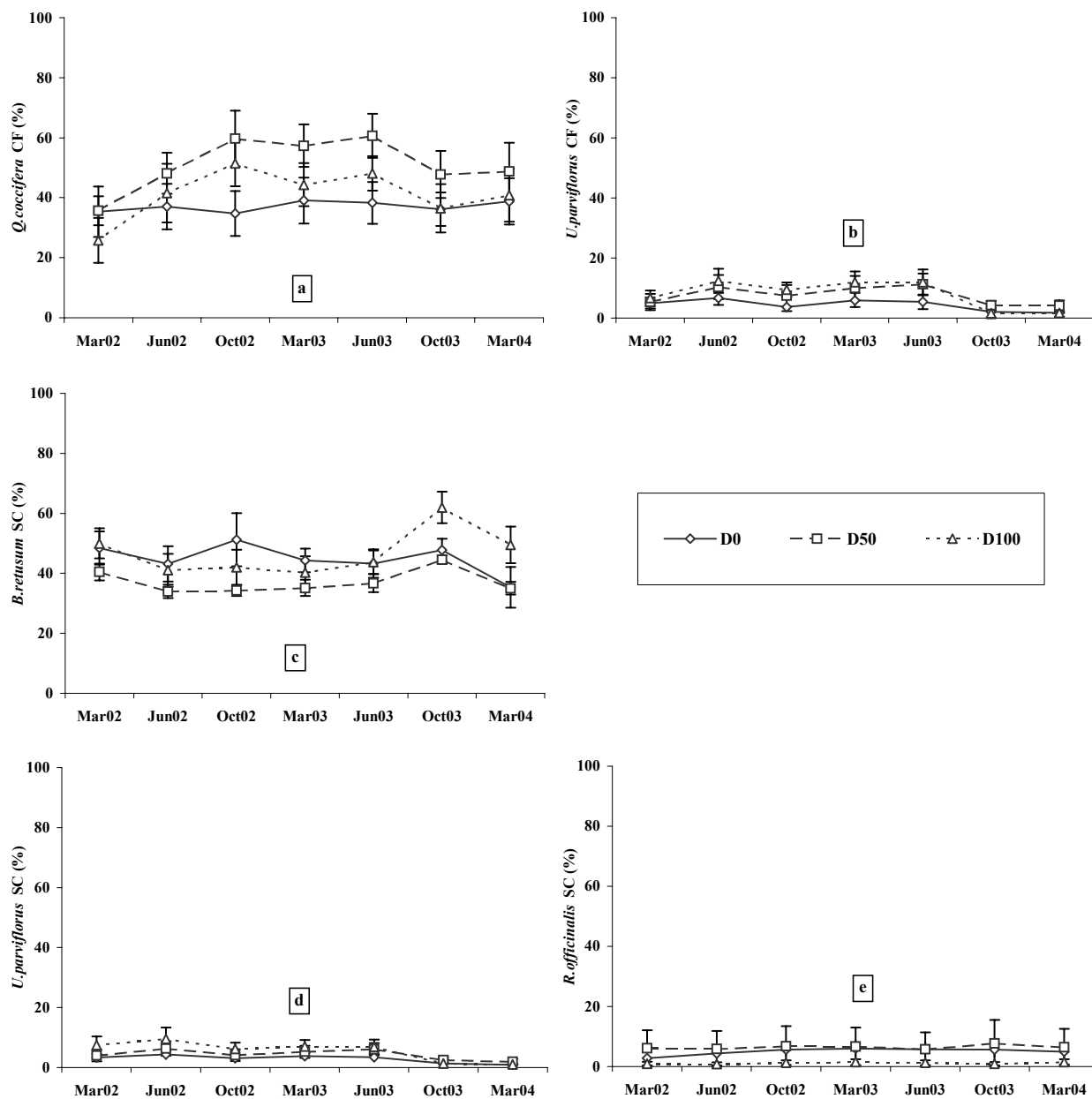


Figure 5. Species cover and SC evolutions during the two years after amendment for D0 (D0), D50 (50 Mg.ha⁻¹) and D100 (100 Mg.ha⁻¹) rates of compost. Mean \pm SE (N=4).

Compost at D50 improved *R. officinalis* and *U. parviflorus* SC and cover. *U. parviflorus* was less affected by crushing because all covers were similar just after amendment. Although *U. parviflorus* is an obligate seeder, Baeza *et al.* (2003) showed that when it is subjected to a disturbance such as clearing (partial loss of its aerial biomass), its aerial part can regenerate by vegetative growth. The compost fertilization effect might have played a part in this species' cover increase. As *U. parviflorus* is a Legume, it can perform atmospheric N₂ fixation (Gimeno-Garcia *et al.*, 2001) and compost N inputs are not of major importance for its fertilization. Thus, as the compost supplies a lot of P to the system, this element could be the one that caused the increased cover of *U. parviflorus* on amended plots. However, Baeza *et al.*, (2003) also showed that *U. parviflorus* could develop an overcompensation effect after the loss of phytomass, and consequently increase its cover. Thus, the crushing effect of amendment could also be responsible for the increase in this species' cover.

The increase of *U. parviflorus* and *R. officinalis* cover on D50 should be responsible for the increasing dominance of woody species versus herbs on these plots. As their covers increase, the shade increases on D50, which may have limited *B. retusum* development and colonization. Competition for light is very important in multiple-layer communities, and the abundance of perennial herbs is inversely related to the amount of woody cover (Calvo *et al.*, 2002a). On D100, *U. parviflorus* and *R. officinalis* have opposite reactions: *R. officinalis* SC decreases on these plots, whereas it increases for *U. parviflorus*. *R. officinalis* may be more sensible to crushing than *U. parviflorus*, and so did not benefit from the compost fertilizing effect on D100.

The cover decrease in October 2003 of both *Q. coccifera* and seeders on amended plots (especially on D100) could be related to the summer 2003 drought. The development of young individuals on amended plots after crushing should have induced a higher population sensitivity to drought. *Q. coccifera* sprouts have a greater proportion of leaf (Cañellas and San Miguel, 1998; Montès *et al.*, 2004), which implies a higher evapotranspiration rate. Similarly, the superficial rooting of seedlings may have limited their access to deep soil water resources. Moreover, for seeder species, the vulnerability to disturbance during regeneration (once germination has begun) is a particularly crucial issue for Mediterranean climate plants since unseasonal rains, or early rains followed by extended dry periods are common (Mooney and Hobbs, 1986). After the summer 2003 drought, *U. parviflorus* cover decreased proportionally to compost rate, D100 cover reaching values below the D0 values. Fertilization has been shown to frequently increase plant sensitivity to drought by increasing the size of transpiring

organs (Van Den Driessche, 1984). Moreover, the compost mulch deposited on the soil could have induced the development of superficial roots more sensitive to drought. Archibold (1995) reports that the distribution of roots is often determined by the availability of nutrients in the soil, and that rooting is particularly dense where organic matter has accumulated.

In contrast, in October 2003, the resprouter group's dominance on D100 was increased due to the rapidity with which the herb *B. retusum* recovered after summer drought, when living conditions become favourable again. Similarly, Caturla *et al.* (2000) noted a second increase of *B. retusum* green biomass in autumn, after the onset of rainfall.

Two years after amendment, compost begins to improve seeder species cover on D50. Their reaction delay could be linked with the production of seeds, whose germination is sensitive to fertilization, implying a complete reproduction cycle. Moreover, the severe drought of the year 2003, which began in May 2003, could have blocked the development of seedlings, and the compost effects appeared after rainy autumn months had recreated favourable conditions of germination and growth. However, it is more likely that seeders have begun to colonize the compost mulch deposited on the subsoiling lines crossing the experimental plots. As these subsoiling lines are nearly free of vegetation, the heliophilous seeder species could be able to colonize them, as compost may constitute a nutrient-rich substrate for the newly-produced seed germination. Moreover, obligate seeders have low demands in terms of soil depth, and readily establish on most substrates (Keeley, 1986). Thus, in garrigues dominated by *Q. coccifera*, compost mulch could facilitate seeder species' establishment between the patches covered by *Q. coccifera*, where the shallow soil is almost bare (Le Houerou, 1973). Similarly to seeders, in October 2003 and March 2004, *B. retusum* SC became significantly higher on D100 compared to other treatments. This species colonizes the 5 to 10 upper cm of the soil with its roots (Caturla *et al.*, 2000; Maestre *et al.*, 2003), and so is able to colonize step by step the compost mulch deposited on the subsoiling lines. Juhren (1966) reports that where the grass cover is thin, as it is on garrigues, *Cistus* seedlings are found on the bare spots whenever a seed source is present. In contrast, *Q. coccifera* has a deep rooting system (Cañellas and San Miguel, 1998), which does not allow it to colonize this new space. Safe sites for obligate resprouters are deep soils or fissures in the substrate with underground water available during the summer drought (Keeley, 1986).

Conclusion

At the beginning of the experiment, compost favoured the two dominant resprouting species because amendment involved a disturbance by crushing. Compost had also a fertilizing effect on *U. parviflorus* that increased its cover, but also its sensitivity to drought. Then, two years after amendment, the compost mulch improved *B. retusum* and seeder covers, especially *R. officinalis* and maybe some *Cistus*, in areas previously unfavourable to plant development. It is very likely that *Q. coccifera* does not benefit either from compost fertilizing effects, or from its mulch effect. In consequence, if this compost beneficial effect on seeders and *B. retusum* remains the following years, it could progressively decrease the domination of *Q. coccifera*. In addition, by increasing functional group diversity, amendment should improve ecosystem functions like primary production, nutrient cycling, as well as various measurements of stability (Madritch and Hunter, 2003).

Aknowledgements

This research was commissioned by the Conseil Général des Bouches-du-Rhône (France), and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the French Rhône-Méditerranée-Corse Water Agency.

Résultats

Le passage répété du tracteur sur les parcelles amendées a écrasé la végétation et induit une diminution de son recouvrement, surtout sur D100 où le tracteur a circulé deux fois plus pour épandre le double de compost. La flexibilité plus grande des espèces herbacées face à l'écrasement a permis une augmentation de leur recouvrement, lequel a diminué sur D100. Dès juin 2002 pour D50 et mars 2003 pour D100 la végétation sur parcelles amendées a retrouvé un recouvrement du même ordre que sur parcelles témoin, et même supérieur sur D50. Ceci montre la forte capacité de la végétation de garrigue à recoloniser l'espace après perturbation. Cette recolonisation après écrasement est majoritairement due aux espèces qui rejettent de souche, surtout sur D100 : ces espèces ont développé un mécanisme de rétroaction positive après le passage du feu qui se traduit par l'occupation rapide de l'espace avant que les espèces semencières ne germent. D'ailleurs, dès le mois d'octobre 2002, la progression de la végétation se stabilise suggérant que tout l'espace disponible créé par l'écrasement a été colonisé.

Le compost à D50 a stimulé le recouvrement de *Rosmarinus officinalis* (N, P) et d'*U. parviflorus* (P), ce qui dénote un effet fertilisant sur ces deux espèces. Il semble que *U. parviflorus* a été moins sensible à l'écrasement que *R. officinalis*, du fait de sa capacité à régénérer après la perte partielle de biomasse aérienne. Le développement de ces deux espèces est probablement responsable de la dominance accrue des ligneux sur D50.

Deux ans après épandage, les espèces semencières commencent à bénéficier de l'apport de compost à D50. Ces espèces ont vraisemblablement colonisé la couche de compost déposée sur les lignes de sous-solage traversant les parcelles expérimentales, car elles sont peu exigeantes vis à vis de la profondeur du sol et s'établissent sur des substrats très variés. Similairement, en fin d'expérimentation, il semble que *B. retusum* s'étende sur ces zones initialement défavorables à l'installation des plantes mais rendues fertiles par l'apport de compost à D100.

Finalement, le rajeunissement des populations de plantes induit par l'écrasement a entraîné une plus grande sensibilité de *Q. coccifera* et des semenciers à la sécheresse de 2003 sur D100. De plus, il semble également que l'effet fertilisant du compost sur *U. parviflorus* a diminué sa résistance à la sécheresse sur D100.

Ainsi, deux scénarii se dessinent en fonction des facteurs d'impact majeurs de l'amendement sur la végétation. Sur D100, l'écrasement est le facteur prépondérant et la réponse de la végétation est modélisée majoritairement par cette perturbation. *B. retusum* est le principal

acteur et bénéficiaire au détriment des ligneux. Sur D50, les effets fertilisants sont prédominants vis à vis des semenciers, surtout *U. parviflorus* et *R. officinalis*, puis probablement des cistes avec la colonisation possible des espaces au préalable infertiles avant épandage. On aboutit ici à une augmentation de la diversité fonctionnelle du système garrigue.

Chapitre 4

**UN AMENDEMENT ORGANIQUE EN GARRIGUE PEUT-IL AMELIORER LE FLUX
D'AZOTE ET DE PHOSPHORE ?**

Cet article fait le bilan, deux ans après épandage, du devenir et de la circulation de l'azote et du phosphore au sein du système sol/plante. Dans un premier temps, nous avons déterminé la production de biomasse végétale deux ans après amendement. Les plantes ont été séparées par espèces, traits de vie et stratégies de régénération. Dans un second temps, les stocks d'azote et de phosphore dans les plantes, la litière, l'humus et l'horizon minéral ont été calculés juste après amendement et deux ans après pour estimer les flux de ces deux éléments entre le sol et les plantes.

Can organic amendment improve N and P turnover in a Mediterranean post-fire shrubland ?

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Article soumis le 21 octobre 2004 à Journal of Applied Ecology

Abstract

1. Under the Mediterranean climate, high fire frequency is responsible for soil impoverishment that induces the development of stable pyrophyte vegetation on the most degraded zones and delays the establishment of potential forest species. Organic amendment could alter the established dominance relationships in such communities, according to the capacity of different species to react to an increase in soil fertility level, and consequently could increase nutrient cycling rates, which could improve succession processes.

2. In a seven-year-old garrigue, N and P stocks were calculated for vegetation, litter, humus and organo-mineral A horizon compartments, as well as plant biomass, just after and two years after compost spreading. Three treatments were studied: control, 50 Mg.ha⁻¹ and 100 Mg.ha⁻¹ of fresh co-composted sewage sludge and green wastes.

3. The *Quercus coccifera* garrigue studied showed relatively balanced N and P budgets as early as 7 years after the last fire, which highlights in a new way the rapid stable state reached by this plant community after the occurrence of fire.

4. Compost amendment durably increased N, and especially P stocks in nutrient releasing reserves of soil / plant system (litter and humus), while it had a limited effect on the organo-mineral A horizon.

5. The improvement of vegetation productivity after organic amendment differed according to the plant's life-traits, maximum increase rates corresponding to herb dominant species, while lower rates corresponded to woody species.

6. Two time-scales were apparent for possible improvement effect of plants on N and P turnover. Seeder and herb species rapidly accumulated N and P from compost, especially *Cistus albidus*. The resprouter *Quercus coccifera*, which is disturbance-adapted, was more sensitive to the crushing effect of amendment than to the fertilization effect. Amendment rejuvenated the population and delayed possible effects on N and P turnover until fresh litter can be provided.

7. After amendment, as P leaf stocks of *Cistus albidus* equal those of *Quercus coccifera*, this species could reach the dominant *Q. coccifera* level of importance for ecosystem P cycling. First, this puts in perspective the common cover- or biomass-based definition of one species' dominance in a plant community. Second, it implies that slight alterations of this species' presence in this community could have major repercussions on the functioning of the ecosystem.

Keywords: *Brachypodium retusum* Pers., *Cistus albidus* L., life-forms, nutrient cycling, *Quercus coccifera* L., resprouters, seeders, crushing.

Introduction

The Mediterranean climate is characterized by long dry summers, as well as frequent strong and dry winds. Both parameters favor recurrent forest fires (Scarascia-Mugnozza *et al.* 2000 ; De Luis *et al.* 2001). High disturbance frequency, increasing with anthropization, have induced the development of a disturbance-tolerant and pyrophyte vegetation on the most degraded zones, such as *Quercus coccifera* garrigues (Le Houerou 1973; Barbero 1990). Indeed, after fire, the previous plant community re-establishes itself by an autosuccession process (Ferran & Vallero 1998). Two main regeneration strategies can be observed: resprouting (vegetative reproduction) and seeding (sexual reproduction) (Baeza *et al.* 2003). Resprouters regenerate by recruitment of new shoots from above- or underground organs after the removal or damage of aboveground parts (Lloret & Vilà 1997). Seeders regenerate by seedling recruitment from a dormant seed bank in the soil (Calvo, Tarrega & De Luis 2002). The evolution of such plant communities after fire is characterized by a relative ecological and floristic stability (Lepart & Escarré 1983). Moreover, after the first phase of re-establishment, the vegetation reaches a stabilization phase, as plant cover and biomass are following a logarithmic function (Ferran & Vallejo 1998). In the latter study, *Brachypodium retusum* Pers. and *Quercus coccifera* L. covers reach a steady state 6 years after fire. In the

first stage of the succession, *Quercus coccifera* rapidly occupy the open space by vegetative multiplication and consequently obstruct the other species installation (Lepart & Escarré 1983). Thus, *Quercus coccifera* garrigue can be considered as a blocking stage of succession (Lepart & Escarré 1983; Quézel & Médail 2003), or even as a pseudo-climax (Cañellas & San Miguel 1998).

The violent precipitations that occur in the Mediterranean region (Scarascia-Mugnozza *et al.* 2000) added to the elimination of plant protecting cover by fires induce soil impoverishment and thinning. Thus, the Mediterranean soils are often deficient in organic matter, nitrogen and phosphorus (Archibold 1995), and have a low water availability (Le Houerou 1973). Consequently, the most competitive plant species in such ecosystems are adapted to low resource availability. Indeed, species have traits that allow them to effectively function for long periods under stress (Tenhunen *et al.* 1984).

Tilman (1984) has shown nutrient fertilization, especially N, to have a significant impact on the relative abundance of dominant species. Moreover, an increase in nutrient disponibility leads to species with low maximum growth rates and low biomass loss rates being replaced by species with high maximum growth rates and high biomass loss rates (Berendse 1998). Yet, compost amendment has been frequently shown to increase soil fertility (Caravaca *et al.* 2002; Martinez *et al.* 2003a), plant biomass (Guerrero *et al.* 2001; Moreno-Peñaranda, Lloret & Alcañiz 2004) and nutrition (Moreno *et al.* 1996). Could a compost amendment increase N and P turnover in a Mediterranean garrigue and decrease the dominance of low-resources adapted species, and by this way decrease the blocking effect on plant succession process ?

Plant biomass assessment is relevant for the evaluation of the productivity of ecosystems, and their cycling of nutrients (Cañellas & San Miguel 2000). Similarly, aboveground litter plays a fundamental role in the nutrient turnover and in the transfer of energy between plants and soil. It contributes to soil humus formation. Both above ground litter and humus are the sources of the nutrients accumulated in the uppermost layers of the soil (Santa Regina, Leonardi & Rapp 2001).

Natural ecosystems studies frequently only consider changes in plant species composition, whereas in many cases the relevant soil and plant processes have not been monitored (Berendse 1998). In this work, we first studied the above-ground biomass accumulation two years after a sewage-sludge compost amendment, in a 7 years old *Quercus coccifera* garrigue. Secondly, the N and P stocks in plants, litter, humus, and organo-mineral A horizon were monitored, just after amendment and two years after amendment, in order to study the N and P fluxes between soil and plants. Plants were segregated by species, life-forms and

regeneration strategies. N was chosen because of its critical importance in plant metabolism, due to its central role in the biochemistry and photobiology of photosynthesis (Rundel 1988). Likewise, P participates to the biosynthesis of plant essential constituents, such as nucleotides and phospholipids (Martin-Prével, Gagnard & Gautier 1984).

Material and methods

Study site and experimental design

The experiment was carried out on 6000 m² in the plateau of Arbois (Southern Provence, France; 5°18'6''E – 43°29'10''N in WSG-84 Norm) at 240 m above sea level and under Mediterranean climatic conditions. The soil was a silty-clayey chalky rendzina, with a high percentage of stones (77 %) and a low average depth (around 24 cm). The mean bulk density of the soil was 1.4 (\pm 0.09). The last fire occurred in June 1995 and the site was colonized by Mediterranean sclerophyllous vegetation, with a 70 % total cover; *Quercus coccifera* and *Brachypodium retusum* being the two dominant species. This natural vegetation belongs to the hoalm oak (*Quercus ilex* L.) succession series (Quézel & Médail 2003).

Compost was surface applied in January 2002. It was put in a tipper-wagon pulled by a tractor, which went over each plot, step by step, on length lines. The experimental design was a complete randomized block of twelve plots of 500 m². Four plots did not receive any compost (D0=control), four received 50 Mg.ha⁻¹ (D50), and four 100 Mg.ha⁻¹ of fresh compost (D100). Inside each 500-m² plot, a subsoiling line was remaining from an ancient plantation done on the site in 1970. These 1-m wide subsoiling lines were length-crossing the plots. Their percentage of outcropping stones was very elevated and consequently, their vegetation cover was very low.

Compost

The compost was produced by a local company (Biotechna, Ensues, Southern Provence, France) and is certified conform to the French norm on composts made with materials of water treatment origin (NF U 44-095 2002). This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark

Table 1: Organo-mineral A horizon (0-24 cm: maximal depth; N=12) and compost (N=3) physico-chemical characteristics. DM: dry matter. FM: Fresh Mass.

| Parameter | Soil | | Compost | |
|----------------------------------|---------------|---|---------------|---|
| | Mean (SE) | Allowed French limit value before sewage sludge amendment | Mean (SE) | Allowed French limit value (08/01/1998) |
| pH _{H2O} | 7.34 (0.008) | | 7.7 (0.05) | |
| Humidity (% FM) | | | 4.8 (0.29) | |
| CEC (meq.100 g ⁻¹) | 23.12 (0.31) | | | |
| Total Calcareous (%DM) | 4.17 (0.13) | | | |
| OM (% DM) | 7.58 (0.12) | | 46.8 (2.74) | |
| total N (% DM) | 0.36 (0.005) | | 2.03 (0.03) | |
| C/N | 12.42 (0.09) | | 13.4 (0.78) | |
| total P (% DM) | 0.037 (0.001) | | 3.24 (0.03) | |
| Exchangeable P (ppm) | 23.3 (0.35) | | 2514.8 (7.82) | |
| Copper (mg.kg ⁻¹ DM) | 19.8 (0.14) | 100 | 144.1 (0.84) | 1000 |
| Zinc (mg.kg ⁻¹ DM) | 78.2 (0.24) | 300 | 265.0 (5.49) | 3000 |
| Cadmium (mg.kg ⁻¹ DM) | 0.31 (0.002) | 2 | 0.8 (0.0) | 15 |
| Chrome (mg.kg ⁻¹ DM) | 67.3 (0.33) | 150 | 27.1 (0.65) | 1000 |
| Mercury (mg.kg ⁻¹ DM) | 0.06 (0.001) | 1 | 0.86 (0.06) | 10 |
| Nickel (mg.kg ⁻¹ DM) | 45.3 (0.17) | 50 | 16.5 (0.23) | 200 |
| Lead (mg.kg ⁻¹ DM) | 43.1 (0.26) | 100 | 57.3 (2.53) | 800 |

pieces and stored in swathes. The swathes were mixed several times in the next 6 months to promote organic matter humification. The final compost met the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals. Soil and compost characteristics before amendment are shown in Table 1.

Field procedures

Soil and organic horizon

Organo-mineral A horizon and humiferous episolum were sampled at two dates: March 2002, one month and a half after amendment, and March 2004, approximately two years after amendment. All humiferous episolum was collected on a 20 x 20 cm square. Then, all accessible organo-mineral A horizon down to bedrock was collected with a soil borer. Indeed, in Mediterranean areas, the soil horizons can not be distinguished. Each analysed organo-mineral A horizon and humiferous episolum sample was a mix of 3 samples randomly collected on each 500-m² plot. Before analysis, samples of humiferous episolum were 2-mm mesh sieved, dried and weighed. The fraction > 2 mm containing compost pine barks and wood pieces and coarse litter corresponded to litter compartment. The fraction < 2 mm containing humified organic matter and composted sewage sludge corresponded to humus compartment (granulometric definition).

Plant aerial biomass

Plant aerial biomass was monitored in March 2004, two years after amendment. A non destructive method was chosen, *i.e.* the point intercept method, which used the idea that an infinite number of points placed within a vegetation sward will hit the plant species proportionally to their cover (Jonasson 1983). Practically, a sharp rod, approximating a point, was vertically shifted on a line crossing vegetation and the number of contacts of each plant species with the rod was noted. Jonasson (1988) showed that the number of each contact for one given species is correlated to this species biomass. Furthermore, he found this method to be highly precise for numerous growth patterns of vegetation.

First, we established a relationship between aerial biomass and number of contacts for the 6 studied species, which represent more than 95 % of the site total vegetation cover: *Quercus coccifera* L., *Brachypodium retusum* Pers., *Ulex parviflorus* Pourr., *Cistus albidus* L., *Cistus*

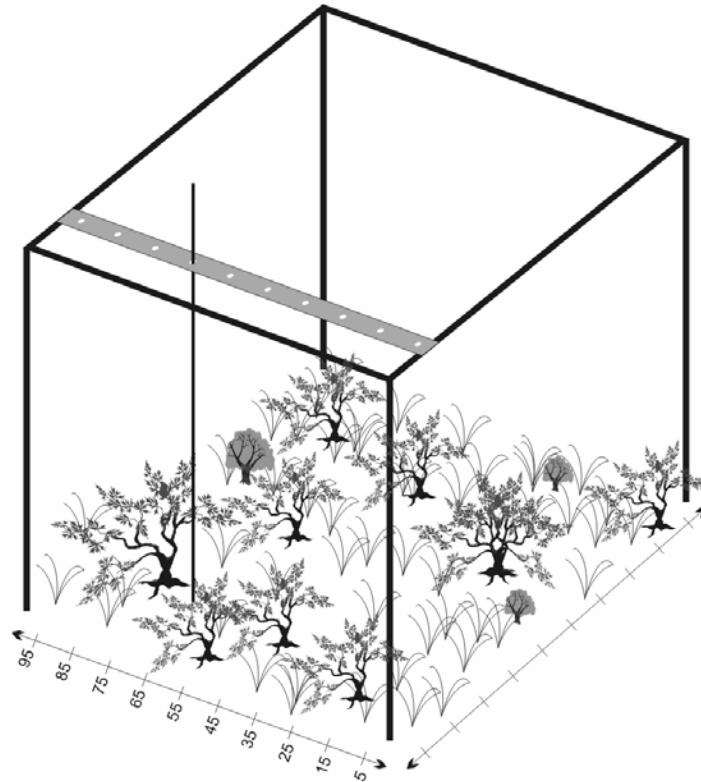


Figure 1. Experimental apparatus used to establish the relationship between the studied species biomass and the number of plant contacts in 1-m² vegetation swards, using the point-intercept method.



Figure 2. Biomass estimation by the point-intercept method on a 5m90 line crossing vegetation of the experimental plots.

salviaefolius L., *Rosmarinus officinalis* L. Fifteen squares of 1-m² were monitored on the experimental site (Fig. 1), outside of the plots. Each square contained 100 rod reading positions, each being representative of a 10 x 10 cm vertical section. At each reading position, the total number of contact with one species was noted, leaf and branches being separated in two distinct categories for woody species with leaves (*Q. coccifera*, *C. albidus* and *C. salviaefolius*, *R. officinalis*). After that, all the above ground vegetation was cut, leaves and branches were separated, oven dried at 70°C (48h) and weighed.

Second, in each plot of 50 x 10 m, we set 4 lines of 5.90 m (4 x 60 reading positions) perpendicularly to the 50 m axis of the plot (Fig. 2). These lines covered perpendicularly the subsoiling line in each plot center. Thus, the calculated biomass on each line corresponded to a 0.6 m² surface.

The data were separated in different categories in order to precise the vegetation reactions to compost amendment. The biomass of the six major species colonizing the experimental site was measured (leaf and branches being separated): *Q. coccifera*, *B. retusum*, *U. parviflorus*, *C. salviaefolius*, *C. albidus* and *R. officinalis*. Secondly, the species were segregated by life forms (woody species and herbs), and by their type of regeneration mechanism (resprouters and seeders).

Plant samples

Plant samples were collected in March 2004, two years after amendment. Three individuals or groups of individuals (*B. retusum*) were randomly cut in each plot and bulked together to constitute one sample per plot. Leaf and branches were separated (*Q. coccifera*, *C. albidus* and *C. salviaefolius*, *R. officinalis*).

Laboratory procedures

Organo-mineral A horizon, humus and litter N and P concentrations (Appendix 2)

The organo-mineral A horizon, humus and litter samples were oven dried at 45°C to constant weight. Soil was sieved through a 2-mm mesh before analysis. Total nitrogen (N) was determined by dry combustion and thermic conductimetry (ISO 13878, AFNOR 1999). Total phosphorus (P) were measured according to NF EN ISO 11885.

Appendix 1. N and P concentrations in leaf and wood of the six studied species two years after amendment (March 2004), on D0 (D0), D50 (amended with 50 Mg.ha⁻¹ of compost) and D100 (amended with 100 Mg.ha⁻¹ of compost) plots. Mean (SE) (N=4), or analysis on bulked samples (N=12).

| | rate | N % DM | | P g/kg DM | |
|-------------------------|------|-------------|------|--------------|------|
| | | Leaf | wood | Leaf | wood |
| <i>Q. coccifera</i> | D0 | 0,92 (0,04) | 0,54 | 0,63 (0,068) | 0,45 |
| | D50 | 0,96 (0,05) | 0,56 | 0,59 (0,012) | 0,47 |
| | D100 | 1,04 (0,06) | 0,59 | 0,69 (0,042) | 0,65 |
| <i>C. albidus</i> | D0 | 1,48 (0,12) | 0,62 | 1,34 (0,08) | 1,13 |
| | D50 | 1,48 (0,15) | 0,84 | 4,00 (0,18) | 0,79 |
| | D100 | 1,63 (0,03) | 0,83 | 3,43 (0,42) | 1,42 |
| <i>B. retusum</i> | D0 | 0,82 (0,07) | | 0,48 (0,015) | |
| | D50 | 1,29 (0,12) | | 1,05 (0,156) | |
| | D100 | 1,32 (0,09) | | 1,56 (0,023) | |
| <i>U. parviflorus</i> | D0 | 1,08 (0,11) | | 0,40 (0,023) | |
| | D50 | 0,95 (0,10) | | 0,77 (0,034) | |
| | D100 | 1,27 (0,13) | | 1,00 (0,064) | |
| <i>R. officinalis</i> | D0 | 1,6 | 0,47 | 1,24 | 0,63 |
| | D50 | 1,54 | 0,51 | 1,49 | 0,76 |
| | D100 | 1,54 | 0,59 | 1,79 | 0,88 |
| <i>C. salviaefolius</i> | D0 | 1,33 | 0,42 | 1,81 | 0,53 |
| | D50 | 1,33 | 0,45 | 3,28 | 0,86 |
| | D100 | 1,57 | 0,56 | 3,33 | 1,19 |

Appendix 2. N and P concentrations in organo-mineral A horizon, humus and litter just after (March 2002), and two years after amendment (March 2004), on D0 (D0), D50 (amended with 50 Mg.ha⁻¹ of compost) and D100 (amended with 100 Mg.ha⁻¹ of compost) plots. Mean (SE) (N=4).

| | rate | organo-mineral A horizon | | humus | | litter | |
|-------------------|------|--------------------------|--------------|-------------|--------------|-------------|--------------|
| | | total N (%) | total P (%) | total N (%) | total P (%) | total N (%) | total P (%) |
| March 2002 | D0 | 0,34 (0,029) | 0,08 (0,007) | 0,7 (0,16) | 0,14 (0,022) | 0,5 (0,16) | 0,06 (0,006) |
| | D50 | 0,40 (0,046) | 0,11 (0,011) | 1,3 (0,08) | 1,49 (0,27) | 1,0 (0,12) | 0,82 (0,12) |
| | D100 | 0,34 (0,023) | 0,10 (0,007) | 1,3 (0,07) | 1,84 (0,46) | 1,0 (0,19) | 0,66 (0,17) |
| March 2004 | D0 | 0,36 (0,029) | 0,09 (0,011) | 0,8 (0,06) | 0,15 (0,012) | 1,1 (0,06) | 0,12 (0,01) |
| | D50 | 0,40 (0,039) | 0,09 (0,015) | 1,5 (0,14) | 2,34 (0,44) | 1,6 (0,04) | 1,17 (0,08) |
| | D100 | 0,41 (0,061) | 0,11 (0,018) | 1,5 (0,08) | 2,61 (0,17) | 1,6 (0,04) | 1,30 (0,10) |

Plant N and P concentrations (Appendix 1)

Plant samples were washed with demineralized water, oven dried at 45°C, crushed (1-mm) and stored for analysis. N concentration was determined according to [Masson & Andrieu \(1996\)](#) modified. Samples (250 mg) were digested in H₂SO₄ and H₂O₂ at 400°C during 3 hours. Then, the solutions were diluted 500 times, filtered at 0.45 µm and analysed by ion chromatography. The eluent used was 26 mmol.L⁻¹ methan sulfonic acid. P concentration was measured by atomic absorption spectrometry after digestion in *aqua regia*.

Statistical analyses

The correlation between plant biomass and number of contacts was tested using different equations. At each sampling date (March 2002 and 2004), one way ANOVA combined with Tukey test ([Zar 1984](#)) were used to make comparisons of the different parameters (plant biomass; plant N and P stocks; organo-mineral A horizon, humus and litter N and P stocks) according to the studied factor (compost rate). Significant level was considered to be 95 %. The softwares Statgraphics plus[®] (version 2.1: Statistical Graphics Corporation, Copyright 1994-1996) and Minitab[®] (release 13 for Windows, 2000, Minitab Inc., USA) were used.

Results

Relation between aerial biomass and number of contacts

For all species, the best fit between aerial biomass and number of contacts was obtained with linear regressions for the fifteen 1-m² vegetation squares (Fig. 3). All correlation coefficients were higher than 0.8, and often higher than 0.9, except for *R. officinalis* ($R^2 = 0.78$).

Plant biomass (Fig. 4)

Two years after amendment (March 2004), total plant biomass was not significantly different on amended plots compared to D0. The biomass of woody species was significantly higher on D50 whereas it was lower on D100. The leaf / branch biomass ratio was significantly higher on D100 due to a significant decrease of the branch biomass of *Q. coccifera*. The *B. retusum* biomass was significantly higher on D100 compared to D50 and D0.

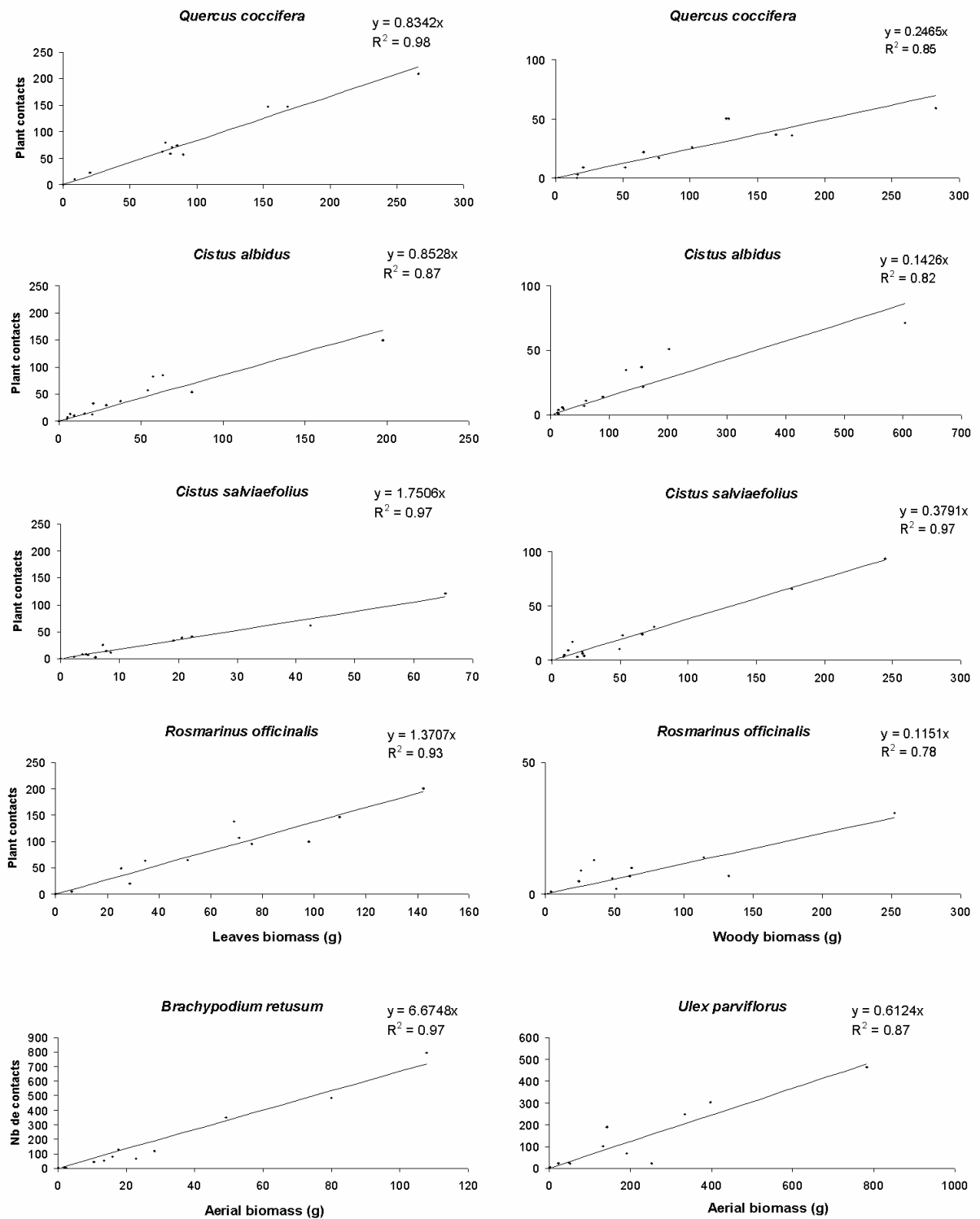


Figure 3. Results of the linear regressions (15 points) monitored between aerial biomass and number of plant contacts, using the point intercept method.

Plant N and P stocks (Fig. 6)

Two years after amendment, compost seemed to have increased N stocked in the vegetation, especially on D50. Similarly, P stocked in plants was significantly higher by a factor 2 on both D50 and D100.

At both rates, compost significantly increased N stock by 30 % and P stock by 100 % in leaves. Compost significantly increased N and P stocks in *B. retusum* proportionally to the applied rate. N stock increased by a factor 2 and 3, and P stock increased by a factor 3 and 7, for D50 and D100, respectively. Otherwise, P stocks were significantly higher on D50 in *C. albidus* leaves. However, N and P stocks in leaves of the other woody species were not affected by compost.

Compost had a significant effect on N stock in *Q. coccifera* branches. N stock of *Q. coccifera* branches was significantly lower on D100 compared to D50 and D0.

Compost effect on organo-mineral A horizon, humus and litter N and P stocks at amendment and two years after amendment (Fig. 5)

Compost had no significant effect on N and P stocks in the organo-mineral A horizon, either in March 2002 (just after amendment), or in March 2004 (two years after amendment).

In March 2002, compost amendment had significantly and strongly increased humus N and P stocks in amended plots ($p=0.003$ and $p=0.048$, respectively for N and P), proportionally to the compost rate (x 15 and x 25 for N, x 85 and x 170 for P, for D50 and D100, respectively). In March 2004, compost effects still persisted but were lower than in March 2002: N and P stocks were significantly higher on amended plots compared to D0 ($p=0.003$ and $p=0.002$, respectively for N and P), but the multiplication factors were 5 times lower for N and 2.5 times lower for P (x 3 and x 5 for N, x 30 and x 60 for P, for D50 and D100, respectively).

In March 2002, litter N and P stocks had increased significantly and proportionally with compost rate ($p=0.024$ and $p=0.031$, respectively for N and P). N stock was multiplied by 6 and 14, and P stock was multiplied by 35 and 65, for D50 and D100, respectively. In March 2004, the compost N and P increasing effects were lower but still significant ($p<0.0001$ for both N and P) (x 4 and x 5 for N, x 30 and x 50 for P, for D50 and D100, respectively).

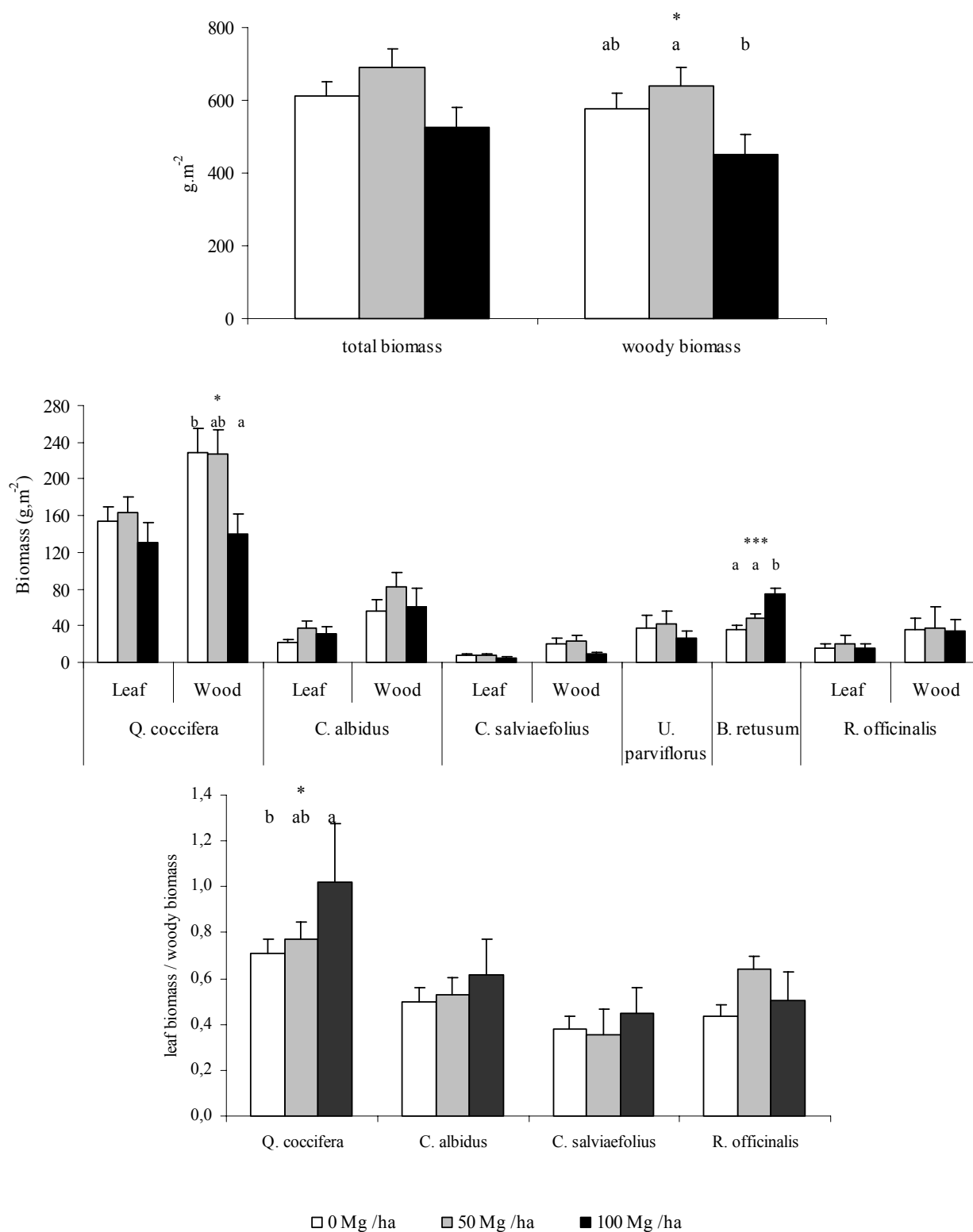


Figure 4. Plant biomass measured on D0 (control), D50 (amended with 50 Mg.ha⁻¹ of compost) and D100 (amended with 100 Mg.ha⁻¹ of compost) plots, for total vegetation, life-forms, and each species (March 2004). Mean (SE) (N=16). Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (a < b). ***p<0.001; **p<0.01; *p<0.05

Patterns of change in organo-mineral A horizon, humus and litter N and P stocks between March 2002 and March 2004 (Fig. 5)

After two years, the dynamics of the two elements are not similar between the 3 treatments. The D0 organo-mineral A horizon is enriched in N (+ 6 %) and P (+ 19 %), as well as the organo-mineral A horizon of D100 (+ 22 % of N and + 5 % of P). However, on D50, N stock remained similar from March 2002 to March 2004, and P stock decreased (- 23 %).

Otherwise, on D0, humus N and P stocks increased by a factor 2 from March 2002 to March 2004. In contrast, humus N and P stocks decreased for both D50 and D100 (- 40 % and - 20 % for N and P, respectively).

Between March 2002 and March 2004, patterns of change in litter N and P evolution differed according to treatment. On D0, both N and P stocks increased by a factor 2. On D50, litter N and P stocks increased by 30 %. In contrast, on D100, litter N stock decreased by 20 %, and P stock was similar from one date to another.

N and P fluxes in soil / plant system (Fig. 5)

In D0, both in March 2002 and 2004, N and P stocks of bulked vegetation, humus and litter, *i.e.* N and P circulating in soil / plant system (root taken separately), represented less than 3 % of soil N and P stocks. N and P stocks of vegetation, humus and litter each represented less than 1 % of organo-mineral A horizon stock for both elements.

The element distribution between compartments changed after amendment. Indeed, in March 2002, N and P stocks of bulked litter and humus reached respectively 15 and 69 % of organo-mineral A horizon N and P stocks in D100, D50 values being twice lower. N and P stocked in humus and litter remained within the same order value in both compartments (around 7-8 % of N and 30-39 % of P in D100).

Two years after amendment (March 2004), the N and P stocks of humus and litter remained very high compared to D0 (6 and 8 % of organo-mineral A horizon N stock, 42 and 58 % of organo-mineral A horizon P stock, for D50 and D100, respectively). These values are lower overall than just after the amendment, except for P on D50, which increased from 31 to 42 % of organo-mineral A horizon P stock from 2002 to 2004, as this element litter stock increased in 2004.



Figure 5. N and P (g.m^{-2}) stocked in the different compartments of soil / plant system following amendment (March 2002) and two years after amendment (March 2004), on D0 (control), D50 (amended with 50 Mg.ha^{-1} of compost) and D100 (amended with 100 Mg.ha^{-1} of compost) plots. Mean (SE) (N=4).

Discussion

N and P stocks in organo-mineral A horizon, humus and litter

Despite a strong increase of N and P stocks in humus and litter after amendment, these elements did not enrich the organo-mineral A horizon, either in March 2002 or in March 2004. This reveals a very low mineralization rate of the used compost, probably due to its high maturity. This could imply that compost spreading preferentially benefits species with superficial rooting, which can absorb nutrients from the zone immediately beneath compost, rather than species like *Q. coccifera*, which has a deep rooting system. Furthermore, the N and P enrichment of humus and litter should increase cycling of these elements in the soil / plant system, at short term for humus elements and at longer term for litter, as most feeding roots can directly dip into litter and humus nutrient reserves (Miller 1984). Two years after amendment, the enhancing effects of compost on N and P cycling in soil / plant system remain, as these element stocks in humus and litter are still higher on amended plots than on D0.

Plant biomass production

Two years after amendment, compost at D50 induced an increase in woody species biomass, whereas it induced a decrease of this parameter on D100. The same pattern was observed for total plant biomass, woody species representing more than 90 % of total plant biomass. Compost amendment is known to increase plant biomass and cover (Moreno *et al.* 1996; Navas, Machin & Navas 1999; Guerrero *et al.* 2001; Martinez *et al.* 2003a; Moreno-Peñarandas *et al.* 2004). Compost supplies N and P to the system and as these nutrients have a plastic role in plant growth (Martin-Prével *et al.* 1984), their increased absorption by plants leads to an increase in their development (Glass 1988). These results are in accordance with what we observed on D50. However, the increases of total plant biomass reported in one year by both Martinez *et al.* (2003a) and Navas *et al.* (1999) are about 200-300 % for similar fertilization rates, whereas in our experiment, D50 woody species biomass increase was only about 12 %. Thus, in comparison to the literature, compost has a low improving effect on plant biomass in our experiment. Six years after fire, the *Q. coccifera* cover has been shown to reach a steady state in a garrigue colonizing degraded lands (Ferran & Vallejo 1998). This dominant species occupies large areas and obstructs other species' development (Lepart &

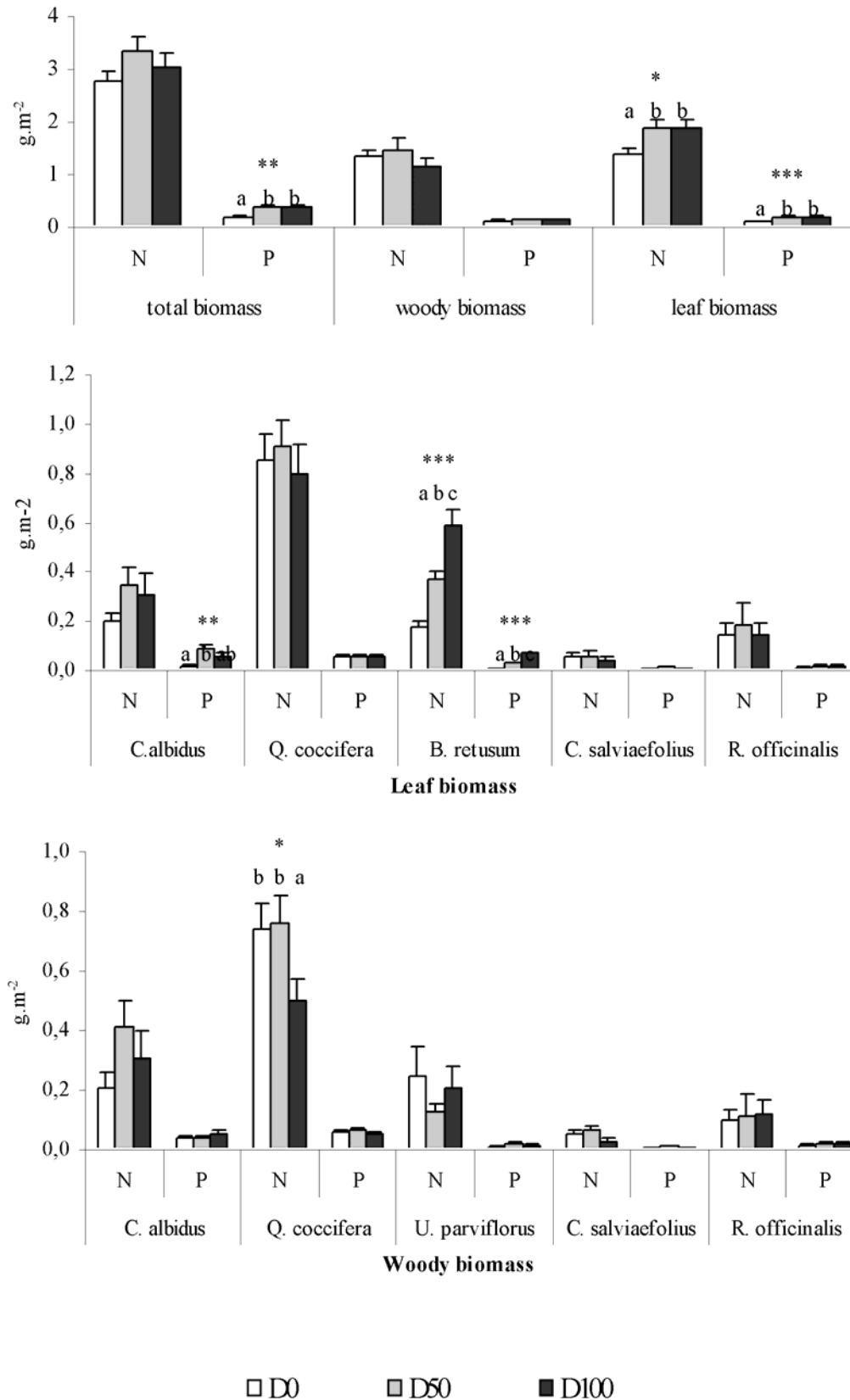


Figure 6. N and P stocked in plant biomass by organs and species two years after amendment (March 2004), on D0 (D0), D50 (amended with 50 Mg.ha⁻¹ of compost) and D100 (amended with 100 Mg.ha⁻¹ of compost) plots. Mean±SE (N=4). Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (a < b). ***p<0.001; **p<0.01; *p<0.05

Escarré 1983). In addition, it is adapted to low resource sites and might not respond to fertilization. This implies a low global response of the plant community to compost fertilization effects.

Furthermore, we found that compost at D100 decreased woody and total plant biomass production. We hypothesize that the crushing of vegetation that occurred when the compost was spread on amended plots had a deleterious effect on woody species, especially on D100, as the spreading machine went past twice. *Q. coccifera* is the dominant woody species at our experimental site (70 % of total plant biomass), and the decrease of its branch biomass on D100 can explain the overall decrease of woody species' biomass production noted on these plots. As a resprouter species, *Q. coccifera* is very competitive in colonizing the space created by disturbances such as crushing (Lloret & Vilà 1997), and it probably rapidly developed some new sprouts. Consequently, the proportion of young *Q. coccifera* individuals is higher on D100 than on D50 and D0, which explains the increase of foliar to woody biomass ratio for this species on D100. Cañellas & San Miguel (1998) note a leaf biomass increase with age up to 6-8 years for *Q. coccifera*. Similarly, Van Den Driessche (1984) reports that young trees have a high proportion of their biomass in foliage, and that this proportion decreases as the proportion of bole and bark increases with age. *B. retusum*, as a herbaceous species is more flexible than woody species and should be less affected by the crushing effect on D100. Thus, this herb follows the results obtained in the literature and its biomass increases with compost application.

N and P stocks in plants

The amendment induces vegetation enrichment in P, and to a lesser degree in N. The compost used was extremely concentrated in P, whereas its N concentration was low. The vegetation enrichment in N and P on amended plots comes from the capture of these two elements by most recyclable parts of the aerial vegetation, *i.e.* herbs apparatus and leaves of woody species. Gosz (1984) reports different decomposition rates for the major plant organs (reproductive > leaf > wood). This increase has two major sources: *C. albidus* and *B. retusum*. *Cistus* species are known to have nutrient accumulation strategies (Carreira & Niell 1992). *C. albidus* is extremely reactive to compost amendment for its nutrition and could act as a sink for P, especially on D50. Moreover, *B. retusum*, as an herb, is highly reactive to an increased nutrient supply (Berendse 1998). This species doubles its biomass on D100 and increases its

N and P absorption, which leads to the capture of these elements in the same order value as the woody *C. albidus*.

Since *U. parviflorus* is a Legume species, it can perform effective atmospheric N₂-fixation that may explain the non-significant results concerning N.

The perennial parts of the vegetation, *i.e.* branches, do not play a part in the overall N and P enrichment of the vegetation. Moreover, *C. albidus* is a malacophyllous species whose leaves have a short life-span (less than one year) compared to sclerophyllous *Q. coccifera* (25-27 months) (Floret *et al.* 1989), and consequently this species should greatly improve the soil / plant system N and P cycling. In addition, *C. albidus* leaves may have a greater decomposition rate than *Q. coccifera* leaves, as sclerophyllous leaves contain greater concentrations of phenolics and lignins, which are not only resistant to decomposition, but may also bind other elements into resistant organic complexes (Archibold 1995). Thus, compost N and P take soil / plant system pathways that increase their overall turnover at short term. Moreover, on D100, the colonization of young *Q. coccifera* individuals should imply high growth rates with resources moved toward leaves preferentially to stocking organs, which could increase overall turnover at longer term.

N and P fluxes in soil / plant system

On D0 plots, from March 2002 to March 2004, N and P stocks naturally doubled in humus and litter compartments. Ranger & Turpault (1999) noted that when a “climatic” equilibrium is reached, losses by drainage strongly decrease and the nutrients budgets (system inputs – system outputs) are balanced, apart from catastrophic events. The ecosystem is adapted to the environmental constraints. In our experiment, the 7-year old garrigue (last fire in June 1995) should have reached a stable N and P cycling estate, which is characterized by slight N and P accumulation. Thus, this study shows that the rapid stabilization of vegetation after disturbance, shown by Ferran & Vallejo (1998) at the plant cover level, also expresses itself at higher ecosystem functioning level, such as N and P cycling processes. Moreover, as N and P stocks also increase in the organo-mineral A horizon, it indicates that some external inputs of these elements (dry and wet deposition, microbial fixation of N; Bonito *et al.* 2003) might have entered the soil / plant system.

While D0 soil N and P stocks increased, organo-mineral A horizon N stock remained stable and P stock decreased on D50 from March 2002 to March 2004. P may leave the D50 organo-mineral A horizon in 2 ways: groundwater leaching or plant root absorption. Groundwater leaching is highly probable, as the site colonization by *Q. coccifera* indicates a very fragmented bedrock (Aubert 1983). On the other hand, this elements' absorption by vegetation can only partly explain the lack of organo-mineral A horizon P on D50, because the vegetation P stocks represent less than 1 % of organo-mineral A horizon P stocks.

Concerning humus, N and P stocks decrease in D50 from 2002 to 2004, while they increase in D0, probably due to the mineralization and leaching down to groundwater.

C. albidus shows higher P capture on D50 and woody biomass increase on these plots. This woody species should have produced a P richer litter, that probably enhanced the P turnover in the soil / plant system. *C. albidus* is highly responsible for this turnover enhancement, as it equals the P stock value in the dominant *Q. coccifera* leaves.

Unlike on D0, D100 N and P stocks in humus decreased between March 2002 and March 2004. *B. retusum* may have played a major part in N and P decrease in humus, as its root colonizes the 5 to 10 upper cm of the soil (Caturla *et al.* 2000; Maestre *et al.* 2003), and as its N and P stocks increased on D100. However, it seems that these element leaching is also responsible for their stock increase in the organo-mineral A horizon.

A consequence of the decrease of woody species biomass on D100 is a decrease in litter production, leading to a decrease in litter N and P stocks from March 2002 to March 2004. *Q. coccifera* young individuals which developed on D100 do not yet provide leaf litter, as *Q. coccifera* leaves fall to litter in their third year (Floret *et al.* 1989). This species being the dominant woody species, this lack of litter may be closely linked to the litter N and P stocks decrease observed from 2002 to 2004.

B. retusum is the only species which reacts to compost amendment by increasing its N and P stocks, but it cannot assure the long-term turnover of such large amounts of N and P supplied by compost, because as a herb it cannot stock these elements in aerial perennial parts as can woody species. Thus, due to the squashing effect of amendment, compost at D100 decreased the turnover rate of N and P in the soil / plant system.

Thus, two patterns of change in the plant community, which are associated with different N and P turnover rates, appear on the two compost rate amended plots, according to the reactions of woody species. On D50, the evolution of N and P turnover is rather controlled by

the seeding *C. albidus*, while on D100, resprouting *Q. coccifera* plays the major role. As the continuity of an ecosystem is associated with stability of the biogeochemical cycle and with balanced nutrient budgets (Ranger & Turpault 1999), the alterations in turnover and fertility on amended plots should lead to future changes in ecosystem functioning.

Aknowledgements

This research was commissioned by the Conseil Général des Bouches-du-Rhône (France), and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the French Rhône-Méditerranée-Corse Water Agency.

Résultats

Le compost accroît fortement et durablement (deux ans) la quantité d'azote et de phosphore stockée dans les fractions humus et litière, ce qui devrait augmenter le recyclage de ces éléments dans le système sol/plante du fait de la capacité des racines à venir puiser les éléments nutritifs directement dans ces deux fractions. Par contre, l'apport de compost n'a pas d'effet significatif sur l'horizon minéral.

L'écrasement de la végétation à l'amendement induit une diminution de la biomasse ligneuse et de la biomasse totale sur D100. *B. retusum*, moins sensible à cette perturbation, voit sa biomasse augmenter proportionnellement à l'apport de compost. Au contraire, *Q. coccifera*, dominant parmi les ligneux, est relativement sensible à l'écrasement puisque deux ans après épandage, sa biomasse ligneuse est plus faible sur D100 que sur D0. Pour cette espèce, le rejet de jeunes pousses après écrasement n'est pas suffisant pour combler le déficit de biomasse engendré par l'écrasement sur une durée de deux ans. Sur D50 par contre, l'effet fertilisant du compost semble prendre le pas sur celui de l'écrasement, d'intensité plus faible, et la production végétale (surtout ligneuse) est plus élevée comparativement au témoin. Cependant, cette augmentation est relativement faible (+12%) et montre que la végétation de garrigue à *Q. coccifera* est un milieu relativement stable où l'espace disponible pour un développement accru de la végétation est limité.

L'apport de compost induit une capture plus importante de la végétation en P et dans une moindre mesure en N. Sur un pas de temps de deux ans, ces éléments sont stockés préférentiellement dans les parties à turn-over rapide, c'est à dire les feuilles des ligneux et les herbes, plutôt que dans les parties pérennes (branches). L'augmentation de l'N et du P stockés dans la végétation est due principalement à *C. albidus* et à *B. retusum*, espèces favorables au recyclage rapide des éléments au sein du système sol/plante. Les stocks foliaires en P de *C. albidus* atteignent les valeurs de ceux de *Q. coccifera*, espèce dominante, ce qui pourrait conférer à ces deux espèces un rôle d'importance égale dans le recyclage du P au sein de l'écosystème. Cela remet en question la définition de la dominance d'une espèce généralement basé sur l'importance relative de la biomasse ou du recouvrement.

Au sein des ligneux, deux évolutions apparaissent en fonction de la dose de compost. Sur D50, le recyclage du N et du P est plutôt contrôlé par le semencier *C. albidus* alors que sur D100, le rôle majeur est tenu par *Q. coccifera*.

La comparaison des stocks de N et de P à deux ans d'intervalle dans les trois fractions du sol permet de mettre en évidence une faible accumulation de N et de P, caractéristique d'un

système en équilibre avec les contraintes climatiques. Ceci montre la rapidité avec laquelle le système de garrigue est capable d'atteindre un état stable après perturbation puisque le dernier feu s'est produit sept ans avant le début de l'expérimentation.

Sur D50, une partie du P de l'humus et de l'horizon minéral a pu être lessivée vers l'eau de la nappe. Cependant, les quantités semblent insuffisantes pour causer des problèmes de pollution sérieux. D'autre part, probablement grâce à *C. albidus*, la litière s'est enrichie en P et en N.

Sur D100, le N et le P de l'humus semblent avoir été lessivés vers l'horizon minéral où ils se sont accumulés. Une faible quantité a pu être absorbée par les racines superficielles de *B. retusum*. Par ailleurs, la production de litière est moindre que sur D0, probablement du fait de la diminution de la biomasse des ligneux sur D100, surtout de celles de *Q. coccifera* dominant dont les jeunes rejets ne fourniront de la litière qu'à l'âge de trois ans.

Ainsi, deux tendances sont mises en évidence selon la dose :

- une stimulation possible du recyclage de N et sûrement de P sur D50 liée à *C. albidus*
- un recyclage de N et P retardé sur D100 du fait du développement de jeunes pousses de *Q. coccifera* dont la production de litière est encore faible.

Chapitre 5

EFFET DU COMPOST SUR LA COLONISATION FONGIQUE ET BACTERIENNE DE LA LITIERE DE *QUERCUS COCCIFERA* DANS UN ECOSYSTEME TERRESTRE MEDITERRANEEN

Cet article concerne l'étude des effets du compost sur la colonisation de la litière de *Q. coccifera* par les microorganismes. *Q. coccifera* est en effet une espèce structurante et importante du paysage de garrigue du bassin méditerranéen et fournit généralement 60 à 70 % de la litière totale. La colonisation par les champignons et les bactéries a été étudiée séparément, du fait du rôle différent de ces deux groupes de microorganismes dans la décomposition de la litière.

Nos objectifs étaient de :

- Déterminer les effets de l'apport de compost sur la colonisation des feuilles de *Q. coccifera* en décomposition par les champignons et les bactéries.
- Hiérarchiser l'importance relative de la contrainte climatique forte en région méditerranéenne (sécheresse) et des effets potentiellement favorables du compost sur la fertilité du sol.
- Préciser le processus de décomposition de la litière au sein d'un écosystème terrestre méditerranéen par une approche discriminant le rôle des bactéries et des champignons.

Compost effect on bacteria and fungi colonization of kermes oak leaf litter

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Article soumis le 15 juillet 2004 à Applied Soil Ecology

Abstract

Terrestrial Mediterranean ecosystems are characterized by low water and organic matter soil concentrations, which become worse with recurrent forest fires. Amendment of biosolids on Mediterranean soils could be a way to recycle them by facilitating ecosystem resilience.

Leaf litter breakdown is a vital process in the functioning of terrestrial ecosystems. A way to determine ecosystem integrity is to study organisms able to mineralize organic matter, *i.e.* fungi and bacteria.

In this study, we determined fungal and bacterial biomass associated with decomposing oak leaves (*Quercus coccifera* L.) in a burnt shrub amended ecosystem during 1 year and a half. Three treatments were studied: control, 50 Mg.ha⁻¹ and 100 Mg.ha⁻¹ of fresh co-composted sewage sludge and green wastes. Despite improving effects of compost on the *Quercus coccifera* litter compartment, especially significant increase of N and P concentrations, bacterial biomass were not affected by organic amendment. However, fungal biomass was depressed on plots amended with 100 Mg.ha⁻¹, and negatively correlated to exchangeable P. Finally, both types of microorganisms followed a marked seasonal dynamics, with peaks of biomass during the wet periods of the year.

Key words: Mediterranean ecosystem, sewage sludge compost, leaf litter, breakdown, ergosterol, bacterial numbers, *Quercus coccifera* L.

Introduction

Soils under Mediterranean climate are undergoing degradations due to water erosion and recurrent fires, which affect their fertility (De Luis *et al.*, 2001). Thus nitrogen, which is often a limiting plant nutrient in soil, is easily lost by volatilisation during wildfires.

Guerrero *et al.* (2001) pointed out that compost addition is a suitable technique for accelerating the natural recovery process of burned soils. Indeed, biosolids can improve the low fertility of soils and constitute an alternative to their landfill disposal. Indeed, it is a source of organic matter and plant nutrients (Brockway, 1983; Martinez *et al.*, 2003a) and it can improve soil physical, chemical and biological properties (McKay and Moffat, 2001; Caravaca *et al.*, 2002). But biosolids present potential environmental risks. Their use can induce heavy metal and organic contaminants accumulation in soils (Brockway, 1983), as well as the discharge of nutrients, especially N and P, to surface and ground waters (Martinez *et al.*, 2003). To decrease risks of heavy metal and salt leaching, the organic matter of biosolids can be stabilized by composting (Garcia *et al.*, 1990; Planquart *et al.*, 1999). In addition, mixing biosolids and other organic wastes with large C/N ratios (such as green wastes) can reduce the rate of nitrogen leaching (McKay and Moffat, 2001). Therefore, compost presents better agronomic potentialities than biosolids.

Compost amendment has been frequently shown to increase soil fertility (Caravaca *et al.*, 2002; Martinez *et al.*, 2003a), plant biomass (Guerrero *et al.*, 2001) and nutrition (Moreno *et al.*, 1996). As a consequence, it could lead to a high nutrient content litter and enhance litter breakdown. Litter breakdown is the principal pathway of nutrients return to soil in an available form to plants (Kavvadias *et al.*, 2001). This aspect is especially important on Mediterranean nutrient poor soils where plant communities rely, to a great extent, on the recycling of litter nutrients (Cañellas and San Miguel, 1998). Factors contributing to the litter breakdown are: soil fertility, litter quality and supply, and climate conditions (Kavvadias *et al.*, 2001).

The litter breakdown process involves three types of organisms: invertebrates, fungi and bacteria. The crucial role of microorganisms is clearly established, and consecutive changes in fungal and bacterial biomass dynamics are a useful way to approach the impact of factors controlling leaf breakdown (Gessner and Chauvet, 1994; Isidorov *et al.*, 2002). Moreover, the soil microbial biomass is often regarded as an early indicator of changes which may occur in

the long term with regard to soil fertility. Likewise, [Wardle et al., \(1999\)](#) showed that microbial biomass responds to addition of fertilizers and of organic residues.

Most studies consider total microbial biomass (Fumigation-incubation, fumigation-extraction, substrate induced respiration and ATP methods; [Martens, 1995](#)), *i.e.* bulked fungi and bacteria ([Borken et al., 2002](#); [Khan and Scullion, 2002](#); [Kunito et al., 2001](#)). However, bacteria and fungi respective role in litter breakdown process is different. Indeed, fungi are able to decompose and assimilate refractory compounds such as lignin or tanins ([Criquet, 1999](#)), although bacteria are not thought to assume notable importance before the leaf material has become partially broken down and decomposed by fungi ([Jensen, 1974](#)). Thus, it is of great interest to study separately their reactions to compost amendment.

In this study, bacterial and fungal biomasses were determined on *Quercus coccifera* L. leaf litter on a Mediterranean burnt area amended with sewage sludge and greenwastes compost.. Indeed kermes oak is one of the most important shrub species in Mediterranean basin, where it covers more than two million hectares and accounts generally for 60-70% of the total litter ([Cañellas and San Miguel, 1998](#)).

Our objectives were to (i) determine the effects of a compost amendment on kermes oak leaf litter colonization by bacteria and fungi, (ii) put in balance the drastic Mediterranean climatic conditions (*e.g.* drought) and the potential improvement of soil fertility by compost, (iii) provide comprehensive data on leaf litter breakdown in terrestrial Mediterranean ecosystems by quantifying separately fungal and bacterial biomass.

Material and methods

Study site and experimental design

The experiment was carried out on 6000 m² in the plateau of Arbois (Southern Provence, France; 5°18'6''E – 43°29'10''N in WSG-84 Geodetic system) at 240 m above sea level and under Mediterranean climatic conditions (Figure 1). The soil was a silty-clayey chalky rendzina, with a high percentage of stones (77 %) and a low average depth (24 cm). The last fire occurred in June 1995 and the site was colonised by typical Mediterranean sclerophyllous vegetation, with a 70 % total cover; *Quercus coccifera* L. and *Brachypodium retusum* Pers. being the two dominant species. This natural vegetation belongs to the hoalm oak (*Quercus ilex* L.) succession series.

Table 1: Organo-mineral A horizon (0-24 cm: maximal depth; N=12) and compost (N=3) physico-chemical characteristics. DM: dry matter. FM: Fresh Mass.

| Parameter | Soil | | Compost | |
|----------------------------------|---------------|---|---------------|---|
| | Mean (SE) | Allowed French limit value before sewage sludge amendment | Mean (SE) | Allowed French limit value (08/01/1998) |
| pH _{H2O} | 7.34 (0.008) | | 7.7 (0.05) | |
| Humidity (% FM) | | | 4.8 (0.29) | |
| CEC (meq.100 g ⁻¹) | 23.12 (0.31) | | | |
| Total Calcareous (%DM) | 4.17 (0.13) | | | |
| OM (% DM) | 7.58 (0.12) | | 46.8 (2.74) | |
| total N (% DM) | 0.36 (0.005) | | 2.03 (0.03) | |
| C/N | 12.42 (0.09) | | 13.4 (0.78) | |
| total P (% DM) | 0.037 (0.001) | | 3.24 (0.03) | |
| Exchangeable P (ppm) | 23.3 (0.35) | | 2514.8 (7.82) | |
| Copper (mg.kg ⁻¹ DM) | 19.8 (0.14) | 100 | 144.1 (0.84) | 1000 |
| Zinc (mg.kg ⁻¹ DM) | 78.2 (0.24) | 300 | 265.0 (5.49) | 3000 |
| Cadmium (mg.kg ⁻¹ DM) | 0.31 (0.002) | 2 | 0.8 (0.0) | 15 |
| Chrome (mg.kg ⁻¹ DM) | 67.3 (0.33) | 150 | 27.1 (0.65) | 1000 |
| Mercury (mg.kg ⁻¹ DM) | 0.06 (0.001) | 1 | 0.86 (0.06) | 10 |
| Nickel (mg.kg ⁻¹ DM) | 45.3 (0.17) | 50 | 16.5 (0.23) | 200 |
| Lead (mg.kg ⁻¹ DM) | 43.1 (0.26) | 100 | 57.3 (2.53) | 800 |

Compost was surface applied in January 2002. The experimental design was a complete randomised block of twelve plots of 500 m². Four plots did not receive any compost (D0=control), four received 50 Mg.ha⁻¹ (D50), and four 100 Mg.ha⁻¹ (D100). The compost was produced by a local company (Biotechna, Ensues, Southern Provence) and is certified conform to the French norm on composts made with materials of water treatment origin (NF U 44-095, 2002). This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 days at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in swathes. The swathes were mixed several times in the next 6 months to promote organic matter humification. The final compost met the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals. Soil and compost characteristics are shown in Table 1.

Field procedures

Leaf litter was sampled 9 times superficially from June 2002 to October 2003, as in the superficial layer decomposed leaves are still entire and leaf litter breakdown is the most efficient (Toutain, 1987). Chemical analysis were performed on this coarse mixed litter (O horizon > 2 mm = O-h^{>2}). Each analysed sample was a mix of 3 samples randomly collected on each plot. Kermes oak entire leaf litter was separated from the O-h^{>2} samples to determine microbial biomass. Green leaves were also collected on 3 bushes per plot to analyse N and P concentrations, at maximal *Quercus coccifera* litterfall in late may (2002 and 2003) (Cañellas and San Miguel, 1998).

Laboratory procedures

Fungal colonization of litter was estimated using their ergosterol concentration, taking advantage of its recent development. Indeed, ergosterol is a fungal indicator that offers an efficient measure of living fungal biomass (Gessner *et al.*, 1991; Gessner and Schmitt, 1996; Cortet *et al.*, 2003). Fifty mg of leaf litter were roughly crushed and lyophilized. Ergosterol was extracted from leaf litter by 30 min refluxing in alcoholic base (Gessner *et al.*, 1991) and purified by solid-phase extraction (Gessner and Schmitt, 1996). Final purification and quantification of ergosterol was achieved by high performance liquid chromatography (HPLC; HP series 1050 chromatograph). The system was run with HPLC-grade methanol at a

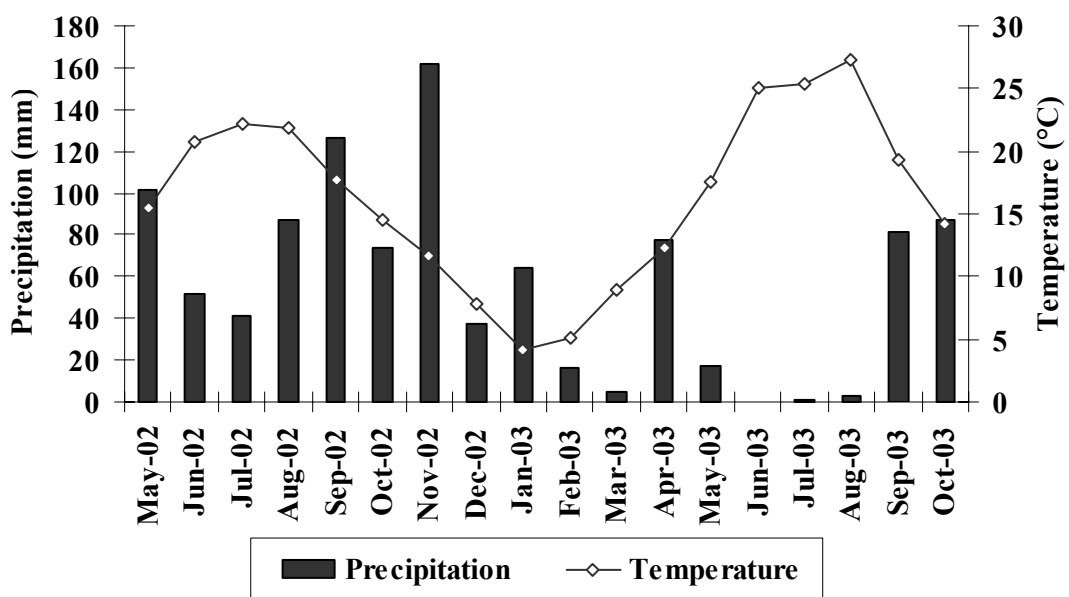


Figure 1. Mean air temperature and precipitation from June 2002 to May 2003 (Météo France).

flow rate of $1.5 \text{ ml}\cdot\text{min}^{-1}$. Ergosterol eluted after 9 min and was detected at 282 nm; peak identity was checked on the basis of retention times of commercial ergosterol (Fluka[®]; > 98 % purity).

Bacterial colonization was determined by counting numbers of bacteria following the general protocol of Porter and Feig (1980) modified by Schallenberg *et al.* (1989). Samples of fresh litter were stored in 2 % formalin before analysis. Bacteria were detached from entire leaves by 2 min probe sonication, according to Buesing and Gessner (2002). Then, 4',6-diamidino-2-phenylindole (DAPI) at a concentration of $5 \text{ mg}\cdot\text{l}^{-1}$ (Baldy *et al.*, 1995) was added for DNA staining. Finally, numbers of bacteria were counted by epifluorescence microscopy.

Soil, compost (< 4 mm) and O-h^{>2} chemistry (total N, total and exchangeable P, total Cu and Zn) were determined using standard procedures (AFNOR, 1999). Exchangeable P was measured by Olsen method (1954). Humidity of *Quercus coccifera* leaf litter was measured by oven drying samples at 60°C during 3 days.

Green leaves of *Quercus coccifera* were washed with demineralised water, oven dried at 40°C and crushed (2mm mesh) in a trace metal free grinder (FOSS TECATOR Sample Mill 1093 Cyclotec). Foliar N concentration was determined according to Masson and Andrieu (1996) modified. Samples (250 mg) were digested in H₂SO₄ and H₂O₂ at 400°C during 3 hours (Bioblock Scientific Digestion Unit 10401). Then, the solutions were diluted 500 times, filtered at 0.45 µm and analysed by ion chromatography (Dionex DX120). The eluent used was 26 mmol.L⁻¹ methan sulfonic acid. Foliar P concentration was measured by atomic absorption spectrometry (VARIAN VISTA Radial) after digestion in *aqua regia*.

Statistical analyses

Two way ANOVA's combined with Tukey tests (Zar, 1984) were used to make comparisons of the different parameters (ergosterol, bacterial number, litter and green leaves chemistry) according to the two studied factors (sampling date and compost rate). Previously, normality and homocedaticity were verified by Shapiro-Wilks and Bartlett tests respectively (Zar, 1984). Data were ln-transformed when test assumptions were not met (*i.e.* bacterial numbers). To relate microbial biomass and chemical characteristics of leaf litter, linear regressions were performed. Significant level was considered to be 95 %. The softwares Statgraphics plus (version 2.1: Statistical Graphics Corporation, © Copyright 1994-1996) and Minitab (release 13 for Windows, 2000, Minitab Inc., USA) were used.

Table 2: Results of two way ANOVA on N and P foliar concentrations in green leaves of *Quercus coccifera* at maximal litterfall (late May) and on O-h^{>2} parameters (only statistically significant results are reported). Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (a < b < c). D0: control plots; D50: plots amended with 50 Mg.ha⁻¹ of compost and D100: plots amended with 100 Mg.ha⁻¹ of compost.

| parameter | factor | F | p | Tukey's test |
|--|--------|--------|--------|---|
| <i>Q.coccifera</i> N concentration in green leaves at maximal litterfall | year | 4.88 | 0.0403 | May02 ^a May03 ^b |
| | rate | 23.55 | <0.001 | D0 ^a D50 ^b D100 ^c |
| <i>Q.coccifera</i> P concentration in green leaves at maximal litterfall | year | 8.66 | 0.0123 | May02 ^a May03 ^b |
| | rate | 4.13 | 0.0433 | D0 ^a D50 ^{ab} D100 ^b |
| <i>Q.coccifera</i> litter humidity | date | 145.43 | <0.001 | June02 ^{bc} July02 ^d Oct02 ^c Dec02 ^f Mar03 ^e Apr03 ^d June03 ^{ab} July03 ^a Oct03 ^c |
| | rate | 10.8 | <0.001 | D0 ^a D50 ^b D100 ^b |
| O-h ^{>2} exchangeable P | year | 53.77 | <0.001 | 2002 ^b 2003 ^a |
| | rate | 115.46 | <0.001 | D0 ^a D50 ^b D100 ^b |
| O-h ^{>2} total P | year | 0.54 | 0.464 | 2002 ^a 2003 ^a |
| | rate | 117.6 | <0.001 | D0 ^a D50 ^b D100 ^b |
| O-h ^{>2} total N | year | 30.06 | <0.001 | 2002 ^a 2003 ^b |
| | rate | 202.2 | <0.001 | D0 ^a D50 ^b D100 ^b |
| O-h ^{>2} total Cu | year | 46.08 | <0.001 | Mar02 ^a Mar03 ^b |
| | rate | 58.52 | <0.001 | D0 ^a D50 ^b D100 ^b |
| O-h ^{>2} total Zn | date | 31.53 | <0.001 | Mar02 ^a Mar03 ^b |
| | rate | 20.69 | <0.001 | D0 ^a D50 ^b D100 ^b |

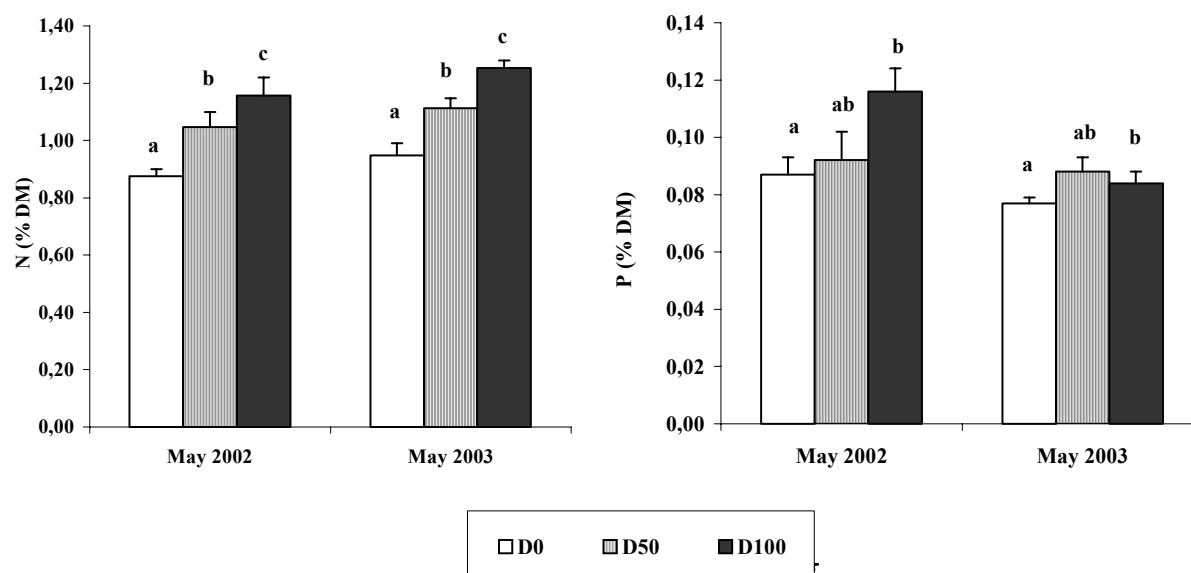


Figure 2: N and P foliar concentrations in green *Quercus coccifera* leaves at maximum litterfall (Late May 2002 and 2003). Bars denote SE (N=4). D0: control plots; D50: plots amended with 50 Mg.ha⁻¹ of compost and D100: plots amended with 100 Mg.ha⁻¹ of compost. Results of the comparison are given by a letter: values that do not differ at the 0.05 level are noted with the same letter (a < b < c).

Results

Quercus coccifera green leaves at maximal litterfall and coarse mixed litter ($O-h^{>2}$)

N and P concentrations in green leaves increased significantly after compost amendment (Table 2, Figure 2). There was no difference between the two compost rates for P, whereas N concentration in green leaves was higher in D100 than D50 both years (2002, 2003). Moreover, N concentration in green leaves increased significantly from May 2002 to May 2003, respectively 5 months and 1.5 year after compost amendment (Table 2). On the opposite, P concentration in green leaves decreased significantly from 2002 to 2003 (Table 2). Compost amendment increased significantly the following physico-chemical parameters of $O-h^{>2}$ (Table 2): $O-h^{>2}$ humidity (Figure 3), exchangeable P (Figure 3), total N (0.92, 1.58, and 1.67 % DM, for D0, D50 and D100 respectively, means of the 9 sampling dates), total Cu (8.75, 75.4 and 69.8 ppm DM, means of the 2 sampling years), and total Zn (50.9, 137.6 and 139.4 ppm DM, means of the 2 sampling years). However, there was no significant difference between the two compost rates (D50 and D100) for these parameters.

Sampling year had also a significant effect on $O-h^{>2}$. Total N and exchangeable P concentrations in $O-h^{>2}$ had a similar evolution as N and P in green leaves from 2002 to 2003 (Table 2). Moreover, N and P concentrations in $O-h^{>2}$ green leaves were positively correlated with concentrations in green leaves ($R=0.45$, $p=0.026$ and $R=0.64$, $p=0.004$ respectively for N and P). Total N in $O-h^{>2}$ increased from 1.3 % DM in 2002 (mean of the 3 treatments) to 1.5 % DM in 2003, whereas exchangeable P decreased from 859 ppm DM in 2002 (mean of the 3 treatments) to 309 ppm DM in 2003 (Figure 3). Total P in $O-h^{>2}$ remained similar (Table 2) from one year (2002, 0.08 % DM) to another (2003, 0.09 % DM).

Humidity of $O-h^{>2}$ was the lowest during the summer 2003 (Figure 3). Spring, summer, and autumn 2003 were exceptionally dry (Figure 1), and as a consequence, October 2003 values were found similar to summer 2002 values.

Microbial biomass of kermes oak leaf litter

Compost amendment had a significant effect on leaf litter colonization by fungi, whereas it did not affect bacterial numbers (Figure 4, Table 3). Indeed, compost at highest rate globally decreased ergosterol concentration in *Quercus coccifera* leaf litter compared to control (159 $\mu\text{g.g}^{-1}$ and 194 $\mu\text{g.g}^{-1}$ DM in D100 and D0 respectively, mean over the studied period), which

Table 3: Results of two way ANOVA on ergosterol concentration and bacterial numbers associated with kermes oak leaf litter. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter ($a < b < c$). D0: control plots; D50: plots amended with 50 Mg.ha⁻¹ of compost and D100: plots amended with 100 Mg.ha⁻¹ of compost.

| parameter | factor | F | p | Tukey's test |
|--------------------|-------------|-------|--------|---|
| ergosterol | date | 10.55 | <0.001 | June02 ^{bc} July02 ^b Oct02 ^{dc} Dec02 ^f Mar03 ^{ef} Apr03 ^{bcd} June03 ^{cde} July03 ^a Oct03 ^{de} |
| | rate | 5.54 | 0.0056 | D0 ^a D50 ^a D100 ^b |
| | date x rate | 1.2 | 0.2845 | |
| number of bacteria | date | 4.52 | 0.0002 | June02 ^{ab} July02 ^a Oct02 ^{bcd} Dec02 ^{abc} Mar03 ^{cd} Apr03 ^d June03 ^{ab} July03 ^a Oct03 ^{ab} |
| | rate | 0.9 | 0.4096 | D0 ^a D50 ^a D100 ^a |
| | date x rate | 0.98 | 0.4852 | |

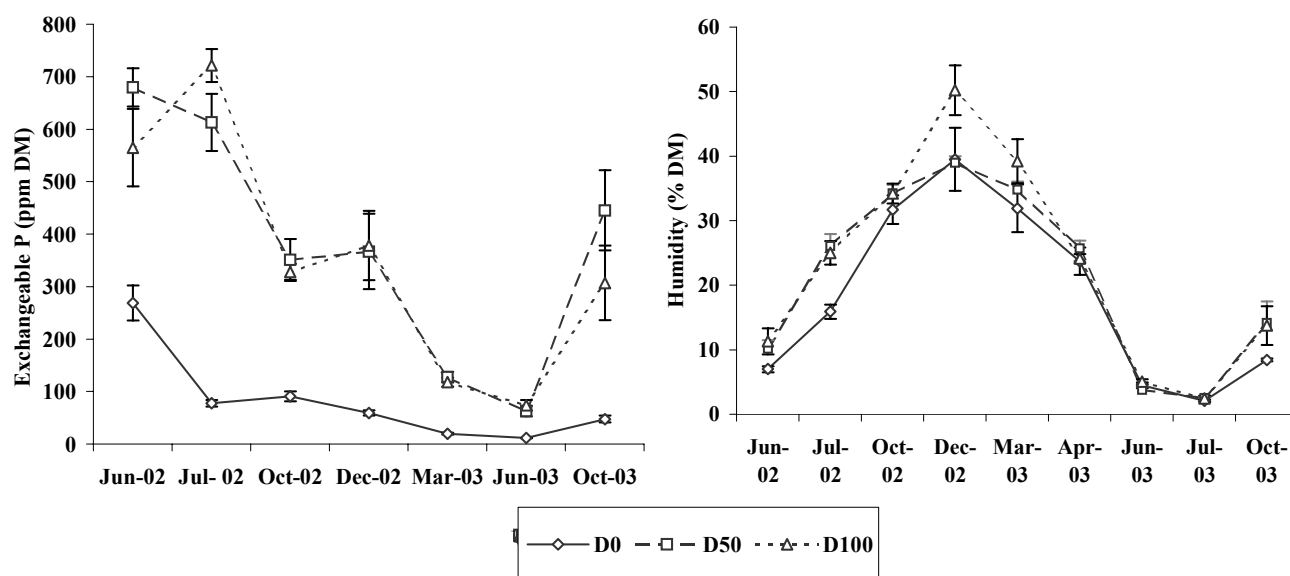


Figure 3: Water and exchangeable P concentrations in the O-h^{>2} from June 2002 to October 2003. Bars denote SE (N=4). D0: control plots; D50: plots amended with 50 Mg.ha⁻¹ of compost and D100: plots amended with 100 Mg.ha⁻¹ of compost.

is clearly settled the second year. Ergosterol concentration for D50 rate ($189 \mu\text{g}\cdot\text{g}^{-1}$ DM) was similar to control. Moreover, ergosterol is negatively correlated to exchangeable phosphorus in $\text{O-h}^{>2}$. However, neither bacterial numbers nor ergosterol are correlated to total P, N, Cu and Zn concentrations (Table 4).

Both ergosterol concentration and bacterial numbers of leaf litter varied significantly according to sampling date within a year. The lowest values corresponded to summers (June and July, both years), when drought is maximal under Mediterranean climate (Figure 1). The mean annual ergosterol concentrations and bacteria numbers were similar during the 2 studied years (Tukey's test, $p=0.4478$ and $p=0.1794$, respectively). In addition, both compartments, bacterial and fungal, were positively correlated with $\text{O-h}^{>2}$ humidity, particularly ergosterol (Table 4). As $\text{O-h}^{>2}$ humidity (month n) is strongly positively correlated ($R=0.76$, $p=0.03$) to precipitations (month $n-1$), the highest ergosterol values corresponded to the highest rainfall of the month before.

Discussion

Microbial biomass of kermes oak leaf litter

Compost amendment decreased significantly kermes oak leaf litter fungal colonization at $100 \text{ Mg}\cdot\text{ha}^{-1}$ rate whereas it had no effect on bacterial colonization. This shows the importance to study separately fungi and bacteria in order to precise the response of microbial communities after a compost amendment. Indeed, in our study the two groups of microorganisms exhibited different reactions. The decrease of ergosterol on D100 amended plots differ from numerous studies which have found enhancing effects of organic amendments on soil microbial biomass (Albiach *et al.*, 2000; Kunito *et al.*, 2001; Ros *et al.*, 2003). However, the ergosterol concentrations observed on *Quercus coccifera* leaf litter of D100 plots remained in the same order values as those of the rare studies attempting to quantify separately bacterial and fungal colonization of Mediterranean litter or soil (Barajas-Aceves *et al.*, 2002; Cortet *et al.*, 2003). On the opposite, litter humidity highly improved *Quercus coccifera* leaf litter colonization. Indeed, *Quercus coccifera* leaf litter moisture was positively correlated both to ergosterol concentrations and bacterial numbers, and fungi were more affected than bacteria. Thus, microbial colonization was the lowest during dry months (June and July) both years, and 2003 values were lower than 2002 values due to exceptional drought. This is in accordance with previous works, such as Papatheodorou *et al.* (2004) who reported seasonal patterns for

Table 4: Matrix of Pearson correlation analysis between ergosterol, numbers of bacteria and O-h^{>2} parameters. *Significant (0.05<p<0.01); ***Highly significant (p<0.001).

| | Ergosterol | Bacterial numbers |
|-------------------------------------|------------|-------------------|
| O-h ^{>2} humidity | R= 0.44*** | R= 0.25* |
| O-h ^{>2} exchangeable P | R= -0.31* | R= -0.13 |
| O-h ^{>2} total P | R= -0.21 | R= -0.02 |
| O-h ^{>2} total N | R= -0.16 | R= 0.04 |
| O-h ^{>2} total Cu | R= -0.39 | R= -0.05 |
| O-h ^{>2} total Zn | R= -0.38 | R= -0.001 |

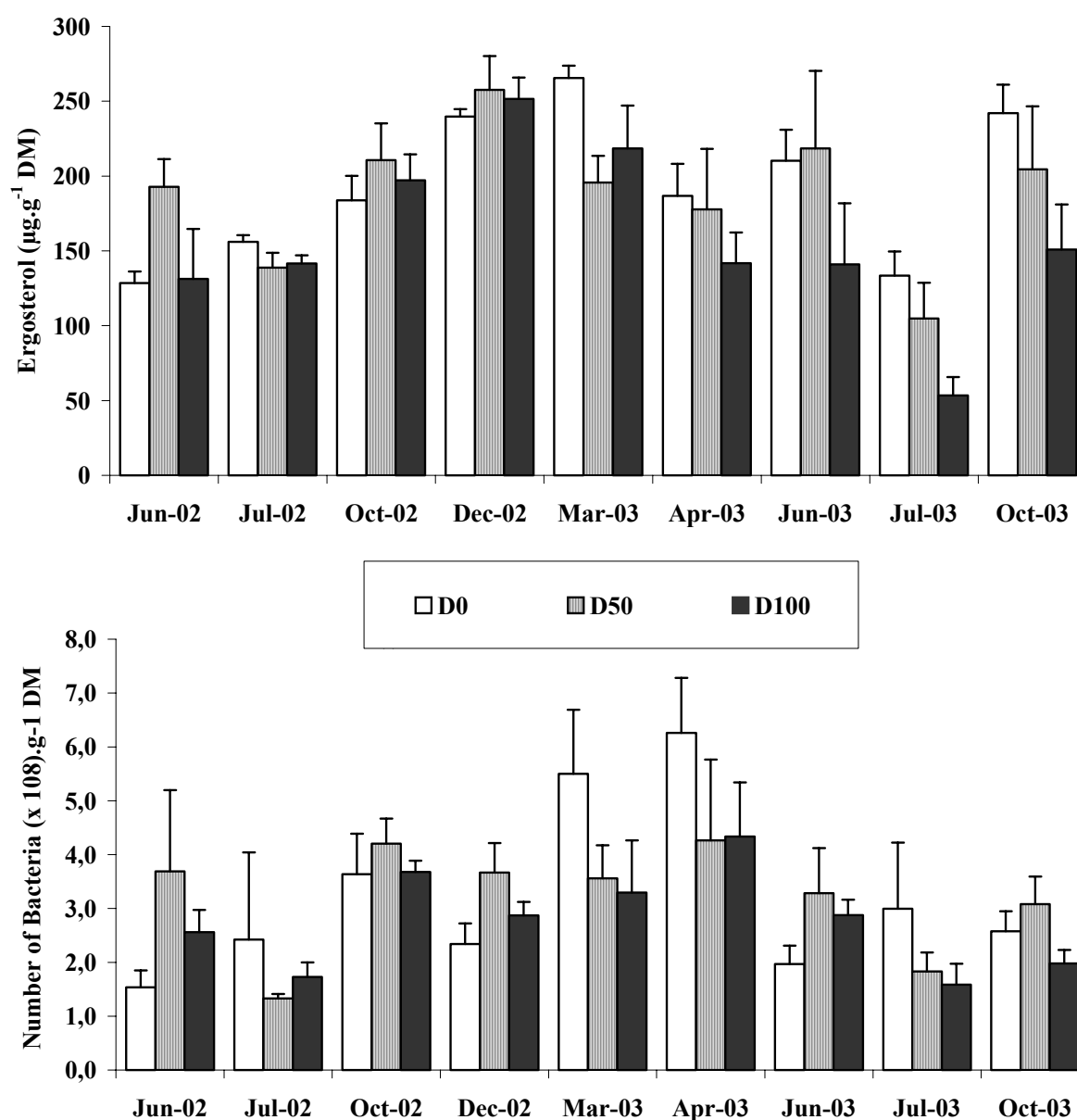


Figure 4: Dynamics of ergosterol concentrations and bacterial numbers associated with leaf litter of *Quercus coccifera* decomposing in control plots (D0), plots amended with 50 Mg.ha⁻¹ (D50) and plots amended with 100 Mg.ha⁻¹ (D100) of compost. Bars denote SE (N=4).

bacterial richness in soil. Likewise, [Criquet et al. \(2000\)](#) and [Barajas-Aceves et al. \(2002\)](#) found that fungal biomass reaches maximal values under humidity conditions in Mediterranean areas.

The compost increased significantly the moisture of *Quercus coccifera* leaf litter in amended plots. This result is in opposition with the depreciating effect of the compost on fungal colonization. Thus, some changes induced by compost amendment may have had strong enough decreasing effects on fungal colonization of kermes oak leaf litter to cancel the increasing moisture effect, even more that drought is one of the major limiting factor in Mediterranean areas.

Total N concentration significantly increased in amended plots (D50 and D100) in kermes oak green leaves and in O-h^{>2}. These two parameters are positively correlated (R=0.45), which suggests that the N increase in green leaves participated to the O-h^{>2} total N enrichment. Indeed, this species generally provides 60-70% of the total litter in garrigue ecosystems ([Cañellas and San Miguel, 1998](#)). As nitrogen is frequently a limiting nutrient in Mediterranean ecosystems ([Archibold, 1995](#)), an increase in O-h^{>2} amended plots improves microbial biomass ([Berg and Söderström, 1979](#)). Moreover, the N enrichment of *Quercus coccifera* litter may decrease its C/N ratio, as well as its polyphenol concentration, leading to an easier breakdown ([Gosz, 1984](#)). However, in our experiment, no significant positive correlation was found between microbial colonization of kermes oak litter and total N concentration in O-h^{>2}. Indeed, and on the contrary to literature, we observed the highest depletion of fungal biomass when total N in O-h^{>2} was the highest: two years after amendment (2003), in D100. Therefore, it is very likely that N accumulates in O-h^{>2} consequently to fungal biomass decrease in D100 plots.

The reduction of fungal colonization at D100 could be the result of a decrease of another limiting factor in Mediterranean ecosystems: P ([Archibold, 1995](#)), which is mostly associated to calcium in inorganic forms on calcareous soils, and then unavailable ([Khanna and Ulrich, 1984](#)). Indeed, [Thirukkumaran and Parkinson \(2002\)](#) reported that microbial variables were unaffected by N addition, whereas an increase of substrate induced respiration (SIR) was found with P fertilization. In addition, [Kwabiah et al., \(2003\)](#) found that phosphorus is the most important quality factor affecting microbial biomass. But, this explanation is not verified for our results. Compost amendment led to an important increase of exchangeable P in O-h^{>2}, as a result of an increase in P concentration in *Quercus coccifera* green leaves (R=0.64).

However, exchangeable P in O-h^{>2} was negatively correlated to fungal colonization (R= -0.31).

In our study, compost amendment induced significant increase in O-h^{>2} total copper and zinc concentrations. As heavy metals are known to affect growth, morphology and metabolism of microorganisms in soils (Dai *et al.*, 2004), Cu and Zn increases could have implied the depreciation of fungal colonization of leaf litter collected on plots amended with 100 Mg.ha⁻¹. However, neither bacterial numbers, nor ergosterol content of leaf litter were correlated to Cu and Zn total concentrations. On one hand, heavy metal total concentrations do not reflect exactly their biological effects and available concentrations in the soil have to be taken into account. On the other hand, Cu accumulates more in plant root (Van Den Driessche, 1984) than in shoot. Thus, the *Quercus coccifera* leaf litter may have poorly been contaminated by this element, explaining the absence of Cu effect on microbial colonization. However, Zn (as Cd, Hg and Ni) is a metal reported to be toxic to microorganisms at lower levels than other metals (Kabata-Pendias and Pendias, 1992). Moreover, this element is considered to be readily soluble relatively to the other heavy metals in soils (Kabata-Pendias and Pendias, 1992). Thus this metal in available form could have deplete fungal colonization of *Quercus coccifera* leaf litter in D100. However, bacteria are known to be less resistant to heavy metals than fungi (Dai *et al.*, 2004) and are not affected by compost amendment in our study. Therefore, the hypothetic depleting effects of Zn on fungal colonization of kermes oak litter have to be put in perspective. Moreover, it is not easy to predict microbial consequences of soil pollution without determining microbial diversity changes under heavy metal contamination, according to species relative sensibility (Dai *et al.*, 2004).

Thus, despites an increase of humidity, N and P concentrations of O-h^{>2} after compost amendment, which should have enhanced this horizon breakdown process, fungal biomass was decreased at D100 rate. As compost contains 57 % of sewage sludge (< 2 mm fraction), which present a higher decomposability (rich in low-molecular dissolved organic carbon and in salts, Agassi *et al.*, 1998) than O-h^{>2} (containing leaves, rich in inhibiting molecules as polyphenols, especially in Mediterranean region, Gershenzon, 1984), we hypothesize that amendment led to a redistribution of fungi from O-h^{>2} to O-h^{<2}. This would induce an accumulation of O-h^{>2} litter and could explain the total N increase that occurred in O-h^{>2} of D100 amended plots from 2002 to 2003. Likewise, Borken *et al.* (2002) showed that compost could induce some redistribution of microbes from one compartment to another. Indeed, they noted that after compost application, microbial biomass decreased in the humiferous

episolum whereas it increased in the mineral soil, the latter being enriched by nutrients released from organic horizon.

Conclusion

In conclusion, compost at D100 induced significant decrease in colonization of *Quercus coccifera* leaf litter by fungi, especially the 2nd year after amendment. Compost had no significant effect on litter colonization by bacteria. Furthermore, compost at D100 increased O-h^{>2} humidity, as well as N and P concentrations, which should have improved this compartment OM breakdown. Compost Zn enrichment could be responsible for the observed fungal biomass decrease, but it should have affected bacteria either. Thus we hypothesize a redistribution of fungi between refractory components of O-h^{>2} to easily decomposable O-h^{<2} containing composted sewage sludge. This could be responsible of leaf litter N accumulation in O-h^{>2}. Therefore, it would be of great interest to study the microbial colonization of O-h^{<2} and mineral soil.

Acknowledgements

This research has been commissioned by the Conseil Général des Bouches-du-Rhône, and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the French Rhône-Méditerranée-Corse Water Agency.

Résultats

Malgré une augmentation du taux d'humidité et des teneurs en N et en P de la fraction organique supérieure à 2mm (litière grossière) après amendement, les processus de décomposition n'ont pas été favorisés. Au contraire, la biomasse fongique de la litière de *Q. coccifera* a diminué sur D100. La teneur en ergostérol, constituant de la membrane des champignons supérieurs, est négativement corrélée à la concentration en P échangeable de la litière grossière. Cependant, aucun effet du compost n'a été décelé sur la biomasse bactérienne. Ces résultats diffèrent des effets stimulants des apports organiques sur la biomasse microbienne mis en évidence par d'autres auteurs.

D'autre part, la colonisation de la litière par les deux groupes de microorganismes montre une dynamique saisonnière marquée, avec des valeurs de biomasse les plus faibles pendant la saison sèche. L'humidité de la litière est fortement et positivement corrélée aux concentrations en ergostérol et au nombre de bactéries, et ce résultat est d'autant plus marqué pour les champignons.

Par ailleurs, l'amendement a induit un enrichissement important de la fraction litière grossière en Cu et Zn totaux. Cet enrichissement ne peut être la cause de la dépréciation de la colonisation fongique sur D100, car les populations bactériennes ne sont pas affectées alors qu'elles sont connues pour être plus sensibles aux effets toxiques des métaux que les champignons.

Par contre, il est possible que l'amendement ait induit une redistribution des champignons de l'horizon organique supérieur à 2 mm, difficilement décomposable car contenant les feuilles riches en polyphénols des espèces méditerranéennes et les écorces de pin subérifiées du compost, vers l'horizon organique inférieur à 2 mm, plus facilement décomposable car contenant des molécules organiques de plus faible poids moléculaire et les sels nutritifs issus des boues d'épuration. Ceci impliquerait une décomposition moindre de la litière de *Q. coccifera* sur D100 et pourrait expliquer l'accumulation de N total observée de 2002 à 2003 dans l'horizon organique supérieur à 2 mm.

Ainsi, il serait fort intéressant de compléter cette étude par celle de la colonisation microbienne des fractions horizon organique inférieur à 2 mm et horizon minéral.

1. Utilisation de compost de boues en garrigue

La maturité du compost choisi permet un relargage lent et durable (2 ans) des nutriments, et limite les risques de pollution des eaux à court terme.

Au cours des deux ans d'expérimentation, les espèces les plus sensibles à la fertilisation (*C. albidus* et *B. retusum*) absorbent efficacement et simultanément les 4 macronutriments majeurs (N, P, K, Mg), ce qui indique que ces éléments sont limitants dans le milieu naturel par rapport à la demande végétale.

Cependant, les quantités de ces éléments apportées par le compost sont plus ou moins importantes et s'épuisent plus ou moins rapidement selon leur solubilité. Le potassium est l'élément le plus soluble et celui qui s'épuise le plus vite. Deux ans après épandage, les réserves stockées dans l'humus issu du compost sont presque épuisées. A l'opposé, le P est apporté en très grande quantité par le compost et semble former des précipités avec le Ca du sol car ses réserves diminuent peu en deux ans. Ceci peut être à l'origine de déséquilibres futurs dans les proportions relatives d'éléments du sol disponibles pour la nutrition végétale. En effet, en fin d'expérimentation, le K sur parcelles amendées revient au niveau de concentration du témoin, limitant probablement la croissance végétale, et risque de limiter la demande en nutriments, donc la consommation de P par les plantes. Les formes disponibles de P risquent donc dans le futur d'être peu utilisées par la végétation et peuvent être exportées vers les eaux souterraines. D'autre part, l'excès de P dans le sol peut bloquer l'absorption d'autres éléments par antagonisme et accentuer la carence en certains éléments en plus faible quantité (K et N).

Le compost, peu contaminé en éléments trace métalliques par rapport aux teneurs réglementaires (arrêté du 8 janvier 1998), a des effets différents sur la contamination du sol en fonction de l'élément considéré.

Malgré le fait que l'apport de compost constitue une source exogène d'ETM pour le milieu garrigue, il constitue un milieu nutritif pour les racines des végétaux (humus) plus dilué en Ni et Cr que le milieu naturel au préalable contaminé en ces deux éléments. Ainsi, à court terme,

cela peut diminuer le possible stress toxique qu'exercent ces deux éléments en forte concentration dans le sol sur les végétaux de la garrigue.

Il semble par ailleurs que l'apport de compost induit un léger lessivage de Cd, qui même si les quantités sont faibles est à signaler du fait de la haute toxicité et de la forte mobilité de ce métal dans les écosystèmes.

Enfin, le compost constitue une source très importante de Cu et surtout de Zn pour le milieu naturel. Le Zn est un élément facilement lessivable et toxique pour les microorganismes à des quantités très faibles par rapport aux autres ETM. L'élévation importante observée de sa concentration dans le milieu naturel après épandage peut à la fois causer des problèmes de contamination des eaux et de blocage de la décomposition de la matière organique. Cet élément constitue le deuxième élément limitant les épandages de compost de boues dans le milieu naturel, en sus du P.

Au vu des fortes quantités en P et en Zn accumulées sur le sol après amendement, des épandages répétés en garrigue ne sont pas envisageables, à moins de diminuer les teneurs des boues en ces deux éléments.

Par ailleurs, la disponibilité à court terme des éléments trace métalliques du sol et du compost semble faible puisque aucune contamination des plantes n'a été mise en évidence. Cependant, la minéralisation progressive du compost peut induire des phénomènes de remobilisation à plus long terme de ces éléments persistants et poser plus tard des problèmes de contamination des producteurs primaires, notamment en Zn et Cu.

2. Fonctionnement du système sol / plantes

Les conséquences de l'apport de compost sur le fonctionnement du système sol / plante sont différentes en fonction de la dose de compost. Celle-ci représente à la fois un gradient dans la quantité de matière organique et d'éléments apportés au sol, mais aussi dans l'intensité d'un facteur de perturbation pour la végétation qui est l'écrasement au moment de l'épandage. Ainsi, l'épandage représente une élévation à la fois du niveau de ressources et de la perturbation.

Le scénario sur D100 semble plutôt défavorable au fonctionnement de l'écosystème pour deux raisons majeures :

- d'une part, l'épandage à D100 a constitué une perturbation importante vis-à-vis de la communauté végétale ligneuse, du fait de l'écrasement des plantes par le tracteur. En conséquence, le compost n'a pas d'effet stimulant sur la production végétale, et les seuls effets observés concernent la réparation de la végétation suite à la destruction engendrée par l'écrasement. Ceci entraîne d'ailleurs une diminution de la production de litière qui ne compense plus la minéralisation.

Ici, ce sont plutôt les capacités importantes de régénération après perturbation de la végétation de garrigue à *Q. coccifera* et *B. retusum* dominants qui sont mises en évidence. Ces deux espèces sont les acteurs principaux de ce phénomène, car capables de rejeter de souche, surtout l'herbacée *B. retusum* qui a peu souffert de l'écrasement. Ces espèces confèrent au système de garrigue une « élasticité de résilience » élevée pour le recouvrement, composante définie comme le taux de rétablissement de l'écosystème pour une propriété donnée après perturbation (Westman 1986).

L'épandage à D100 induit un rajeunissement de la population de *Q. coccifera* et accroît la dominance de cette espèce, renforçant l'installation de la série de végétation régressive. En effet, cette espèce diminue la diversité végétale de la garrigue en limitant ou empêchant l'implantation d'autres espèces grâce à son encombrement spatial, ce qui engendre une phase de blocage, levée par des perturbations ou la sénescence des individus (modèle d'inhibition de Connell et Slatyer, 1977, *in* Van Breemen & Finzi 1998 ; *in* Quézel & Médail 2003).

- d'autre part, l'apport à D100 diminue la colonisation par les champignons de la litière de *Q. coccifera*, espèce qui produit la majeure partie de la litière de la garrigue.

Ceci implique une accumulation possible de cette litière au cours du temps, ainsi qu'une possible réorganisation des communautés microbiennes avec disparition des microorganismes capables de dégrader les substrats peu décomposables caractéristiques de la garrigue au profit d'organismes plus polyvalents. A plus long terme, une fois que la matière organique du compost aura été minéralisée, quelles seront les capacités des communautés microbiennes à se réadapter aux substrats peu décomposables de la garrigue, tels la litière de *Q. coccifera* ?

Ainsi, sur D100, la matière organique et les éléments apportés par le compost et stockés dans l'humus et la litière sont peu mis en circulation dans le système sol / plante, du fait des effets plutôt négatifs de l'épandage sur les deux compartiments actifs dans le recyclage des éléments : les plantes qui produisent la litière, et les microorganismes qui la décomposent. La seule espèce qui bénéficie des effets du compost est *B. retusum*, mais son impact sur le recyclage des éléments du compost est limité du fait de sa faible biomasse et de son incapacité à stocker les éléments absorbés dans des organes aériens pérennes. Cependant, *Q. coccifera* s'est montré réactif à l'apport de compost vis-à-vis de sa nutrition foliaire, et la croissance progressive des rejets induits par l'écrasement, ainsi que l'initiation de leur production de litière à partir de l'âge de 3 ans peut jouer un rôle futur important dans la mise en circulation des éléments apportés par le compost.

Au contraire de D100, le scénario sur D50 semble plutôt favorable vis-à-vis du fonctionnement du système sol / plante.

Les éléments du compost stockés dans l'humus et la litière semblent minéralisés efficacement par les décomposeurs et sont absorbés par la végétation. Toutes les espèces étudiées réagissent positivement à l'apport de compost pour leur nutrition. Ce résultat indique clairement que les espèces de la garrigue sont réactives à une augmentation du niveau de ressources, et que ces espèces, plutôt que d'être adaptées à un milieu où la disponibilité des ressources en nutriments est limitée, sont adaptées à un milieu soumis à des perturbations récurrentes.

Deux espèces se démarquent par leur réactivité plus grande et leur capacité à accumuler les nutriments du compost dans les parties photosynthétiques : *B. retusum* et *C. albidus*. Le recyclage de la totalité de la biomasse foliaire de ces deux espèces chaque année et la décomposabilité importante de la matière végétale produite impliquent une mise en circulation efficace par ces deux espèces des éléments puisés au sein du compost.

C. albidus apparaît ici comme une espèce stratégique de la garrigue vis-à-vis du recyclage des éléments, surtout du P. En effet, les quantités d'éléments qui transitent par ses feuilles sont du même ordre que celles des feuilles de *Q. coccifera* pour une biomasse beaucoup plus faible.

Q. coccifera et *U. parviflorus* ont un effet plus retardé sur les cycles des éléments du fait de la faible décomposabilité de leur litière lignifiée. Ainsi, il se dessine deux stratégies bien

distinctes illustrant l'utilisation complémentaire des ressources qui existe au sein de la garrigue au niveau temporel. Cette utilisation complémentaire existe également au niveau spatial puisque *Q. coccifera* absorbe peu d'éléments comparativement à *C. albidus* mais colonise une surface beaucoup plus importante que ce dernier. L'apport de compost à D50 favorise la diversité fonctionnelle du système de garrigue vis-à-vis de l'utilisation des ressources.

Au niveau spatial, l'hétérogénéité du milieu garrigue favorise la mise en place d'îlots de végétation sur les zones au préalable infertiles qui ont été recouvertes par le compost et sont devenues propices à l'installation des plantes. Les espèces semencières colonisent ces nouveaux espaces non encore marqués par l'omniprésence de *Q. coccifera*. Au sein de ces îlots pourront se produire des changements internes liés à l'arrivée d'autres espèces, à la croissance et aux interactions entre organismes, aux cycles des nutriments, entraînant la mise en place d'une microsuccession locale. Ces changements locaux pourront avoir des effets non négligeables sur la dynamique globale de la végétation de garrigue, en considérant cette dernière comme un processus intégrant les différents niveaux d'hétérogénéité spatiale et temporelle au sein de la communauté végétale, et non pas comme un processus uniforme (Donnegan & Rebertus 1999).

D'autre part, comme la productivité et la disponibilité en ressources d'un site contrôle en partie le retour aux conditions antérieures à la perturbation (White & Jentsch 2001), la stimulation du recyclage des éléments sur D50 liée à la réponse rapide des semenciers et de *B. retusum* devrait améliorer la résilience du système garrigue après feu. De plus, le déséquilibre entre semenciers et espèces à rejet de souche au sein des ligneux s'amenuise et pourrait potentialiser les capacités de résilience du système garrigue. L'augmentation à moyen terme de la production de graines et donc de la diversité génétique, ainsi que l'augmentation de la présence d'espèces à pouvoir de fixation rapide et intense des éléments libérés après incendie participeraient à cette potentialisation de la résilience. De plus, l'écosystème évolue dans le sens d'une complémentarité spatio-temporelle limitant les pertes d'éléments minéralisés après incendies, avec *Cistus albidus* qui fixe rapidement de grandes quantités d'éléments sur des surfaces réduites, alors que *Quercus coccifera* fixe peu d'éléments mais sur des zones très étendues.

Au sein du système garrigue typiquement méditerranéen, caractérisé par un faible niveau de ressources et adapté à la récurrence des incendies, le facteur sécheresse limite les effets du compost sur le système sol / plante. Cela montre le rôle prépondérant du climat dans la dynamique et la structuration des écosystèmes méditerranéens (Quézel et Médail 2003). Suite à la sécheresse de 2003, la productivité (recouvrement), les concentrations en nutriments foliaires, la colonisation de la litière par les microorganismes chutent de manière importante à la fois sur parcelles amendées et sur témoin.

Le compost ne semble pas avoir d'effet protecteur vis-à-vis de la sécheresse lorsqu'il est épandu en surface. Au contraire, il accentue la vulnérabilité des arbustes à la sécheresse vis-à-vis de leur productivité, notamment pour *U. parviflorus*. Ceci s'explique par le rajeunissement de la communauté végétale (écrasement), le développement d'une plus grande surface foliaire et de racines superficielles après épandage. D'autre part, la présence de compost augmente la mortalité des plantes en période de sécheresse, induisant une accumulation ponctuelle de litière. *Q. coccifera* montre les meilleures capacités de résistance à la sécheresse vis-à-vis de son statut nutritionnel, du fait de son système racinaire profond.

3. Caractérisation des réponses des espèces de la garrigue

Sur l'Arbois, au cours des deux ans d'expérimentation, la végétation de garrigue fut soumise au stress hydrique (sécheresse climatique et édaphique), à une augmentation du niveau de ressources minérales (fertilisation par le compost), et à une perturbation de faible intensité (écrasement). L'étude des réactions de la végétation en conditions de coexistence de ces trois types de facteurs, conditionnant les réponses des plantes en fonction de leurs traits physiologiques et biologiques, permet d'élaborer une classification relative des quatre espèces majeures de la garrigue selon le modèle CSR de stratégies végétales de Grime (Grime 1979, in Van Breemen & Finzi 1998 ; in Hodgson *et al.* 1999).

Cette classification est valable pour le stade de garrigue étudié, et non pas au niveau des espèces de la succession végétale dans son ensemble. De plus, dans cette étude, les espèces considérées comme les plus compétitives vis-à-vis des ressources en nutriments seront celles montrant les plus fortes teneurs foliaires en éléments, et non pas celles présentant la plus forte production de biomasse.

L'apport de compost crée un gradient expérimental de ressources qui met en exergue les capacités relatives des plantes pour l'accession aux nutriments, et s'intègre donc dans la

logique d'un gradient de compétition entre espèces. *C. albidus*, qui développe une stratégie d'accumulation des nutriments (Carreira & Niell 1992), est le plus compétitif pour l'absorption des nutriments, suivi par *B. retusum*, puis *U. parviflorus* et *Q. coccifera*.

Face à la sécheresse, *Q. coccifera* se montre le plus tolérant, suivi de *B. retusum*, de *C. albidus* et enfin d'*U. parviflorus*. Ceci est en accord avec les conclusions de Van Breemen & Finzi (1998), qui trouvent que les espèces vérifiant le modèle d'inhibition de Connell et Slatyer, tel *Q. coccifera*, développent également la stratégie de tolérance au stress de Grime, caractérisée par une croissance lente sur les sols pauvres en nutriments et des concentrations foliaires en nutriments faibles. Cette espèce montre donc les meilleures capacités de tolérance à la fois au stress hydrique, et à une limitation en nutriments. D'autre part, les réactions de *C. albidus* face à la sécheresse confirment sa position au pôle compétitif.

Enfin, vis-à-vis de la perturbation créée par l'épandage mécanique, les plus fortes capacités de régénération à court terme après perte de biomasse sont développées par *B. retusum*, suivi d'*U. parviflorus*, puis de *Q. coccifera*, et des autres semenciers (*C. albidus*, *Cistus salviaefolius*, *R. officinalis*). *B. retusum* montre la plus forte amplitude de résilience à l'écrasement, composante définie comme le seuil à partir duquel le retour à l'état initial ne se produit pas (Westman 1986).

La mise en relation de ces résultats permet de placer les deux ligneux *Q. coccifera* (S) et *C. albidus* (C) et l'herbacée *B. retusum* (R) aux trois pôles du modèle de Grime. *U. parviflorus* développe plutôt une stratégie rudérale, mais son positionnement par rapport aux deux autres pôles est mitigé, du fait de ses réactions différentes selon la ressource considérée. En effet, vis-à-vis de la disponibilité en nutriments, il est plutôt stress tolérant, alors qu'il est le plus éloigné de ce pôle pour la disponibilité en eau.

DEUXIEME PARTIE
EPANDAGE DE COMPOST DANS LA PLANTATION

Introduction

Après l'étude des effets d'un amendement organique sur le fonctionnement du stade garrigue à *Q. coccifera*, nous nous intéressons ici aux effets sur le stade de végétation suivant, plus précisément sur l'installation d'espèces arborées naturelles. Les réactions de *Pinus halepensis* (modèle expansionniste), de *Quercus ilex* (modèle de résistance) et de *Quercus pubescens* (modèle de stabilisation), espèces représentant des stades de colonisation successifs potentiels du milieu de garrigue, sont étudiées après apport de compost. Une quatrième espèce, *Pinus pinea*, est également plantée mais est peu représentative des arbres qui colonisent naturellement le site expérimental. Les effets stimulants du compost sur l'installation et le développement de la végétation sont connus. Cependant, peu d'études se sont intéressées aux effets sur des jeunes plants d'arbres *in situ*, spécialement dans des zones méditerranéennes dégradées par le passage répété du feu.

Deux faciès caractérisés par des conditions écologiques différentes sont choisis pour la mise en place de cette étude. *P. halepensis*, *P. pinea* et *Q. ilex* sont plantés sur le plateau plus sec à sol moins profond et moins riche en matière organique, alors que *Q. pubescens* est planté en pente orientée nord, dans des conditions plus humides.

Le compost est apporté 7 mois avant plantation (Mai 2001), au pied de chaque futur plant, en mélange au sol. Les doses sont de 20 et 40 kg de compost brut par plant, soit 8 et 16 tonnes MF par hectare, en comptant 400 plants par hectare.

Les objectifs de cette étude étaient de déterminer les effets de l'apport de compost sur (i) la fertilité du sol, (ii) la survie, la croissance et le statut nutritionnel des plants, et (iii) la contamination en éléments traces du sol et des plants.

Dans un premier temps, les réactions des trois espèces plantées sur le plateau (*Pinus halepensis*, *Pinus pinea*, *Quercus ilex*) à l'apport de compost seront étudiées, puis l'attention se portera sur les réactions de *Quercus pubescens* dans la pente. Enfin, la synthèse générale des résultats obtenus dans la plantation mettra en exergue les points favorables à l'utilisation de compost de boues en plantation forestière pour le reboisement après incendie, ainsi que les limites environnementales d'une telle pratique.

Chapitre 1

**L'UTILISATION DE COMPOST POUR REBOISER DES ZONES MEDITERRANEENNES
INCENDIEES : EFFETS SUR LE SOL ET LES JEUNES PLANTS ARBORES**

**The use of compost to reforest Mediterranean burnt areas :
effects on soil properties and young tree seedlings.**

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Article soumis le 28 octobre 2004 à Applied Vegetation Science (J. Veg. Sci.)

Abstract

Since sewage sludge dumping has been forbidden in EEC, studying new ways of recycling becomes of greater interest. As the Mediterranean area provides a lot of degraded burnt lands to reforest, an organic amendment could improve soil quality and so favour reforestation.

A frequently burnt calcareous Provence's site (France) characterized by soil drought and low organic matter content was chosen. We set a plantation of one-year-tree-seedling of native species (*Quercus ilex*, *Pinus halepensis* and *Pinus pinea*). Fresh co-composted sewage sludge and greenwastes were incorporated into the soil at each seedling stem. Three rates of compost, were used: D0 without compost, D20 and D40 with respectively 20 and 40 kg.m⁻² of compost at the stem of each seedling. The tree density was 400 seedlings.ha⁻¹ and so we really spread 8 and 16 Mg.ha⁻¹ FM of compost. The evolution of soil properties and the development of trees were followed respectively for 3 years (2001-2003) and 2 years (2002-2003).

The compost improved survival of *Quercus ilex* and *Pinus pinea* seedlings in severe drought conditions, increased seedling length and radial growth and NPK nutrition. Amendment increased soil fertility, but available P concentration increased 13 fold in the neighbouring soil of each D40 amended seedlings compared to control, which could be environmentally hazardous. However, amendment did not significantly increase concentrations of Cd, Cr, Ni and Pb in soils or tree seedlings. It increased Cu and Zn total and available concentrations in soils, while foliar Cu and Zn concentrations in the seedlings remained similar in all plots. Limits of compost amendment to reforest Mediterranean areas are discussed.

Key words: Sewage sludge compost; burned Mediterranean area; reforestation; trace metals; *Pinus halepensis*; *Pinus pinea*; *Quercus ilex*.

Introduction

Soils under Mediterranean climate are undergoing degradations due to water erosion and recurrent fires, which affect their fertility (De Luis *et al.*, 2001). The continual removal of weathered rock material has resulted in rather thin and infertile soils which are often deficient in macronutrients (Archibold, 1995). Fire consumes organic matter (above ground vegetation, litter, superficial soil layers) and its nutrients are either volatilized or deposited in the ash (Grogan *et al.*, 2000). Nutrients volatilized, especially N, or not immediately absorbed by plants are lost from the site. Those not immediately absorbed are taken away by erosion or leached into the groundwater. Therefore, in Mediterranean areas, fire and erosion result in the loss of organic matter and nutrients, which decrease soil fertility and has a negative effect on the recovery of the forest vegetation.

Sewage sludge is a source of organic matter and plant nutrients (Brockway, 1983; Martinez *et al.*, 2003a), since it can improve soil physical and chemical properties (Caravaca *et al.*, 2002; McKay and Moffat, 2001). But if sewage sludge recycling seems to be an attractive way to enhance soil potentialities and an alternative to its landfill disposal, it presents potential environmental risks. Its use can induce heavy metal and organic contaminants accumulation in soils (Brockway, 1983), as well as the discharge of nutrients, especially N and P, to surface and ground water (Martinez *et al.*, 2003b). Indeed, sewage sludge amendment increases soil low-molecular dissolved organic compounds (DOC) and soluble salts (*e.g.* carbonates, phosphates from water purification and newly-formed biological breakdown products as NH_4^+ and HCO_3^-) (Agassi *et al.*, 1998).

Composting is a biological process of aerobic decomposition, which degrades labile organic matter to carbon dioxide, water vapour, ammonia, inorganic nutrients to obtain a stable organic material (compost) containing humic-like substances (Senesi, 1989). It may stabilize the organic matter concentration of sewage sludge and by this way decrease the risks of heavy metals and salts leaching (Garcia *et al.*, 1990; Planquart *et al.*, 1999). Moreover, the use of other organic wastes with large C/N ratios (such as green wastes) in mixture with biosolids can reduce the rate of nitrogen mineralization and then decrease its leaching risks (McKay and Moffat, 2001). Indeed, the release of plant available N from organic amendment in the soil is controlled by the balance of N immobilization and mineralization, which in turn, is controlled to a large extent by the C/N ratio of the decomposing organic material (Cambardella *et al.*, 2003).

Thus, improved soil biological properties after compost amendment can, as a consequence, speed plant establishment (Brockway, 1983; Dutch and Wolstenholme, 1994; Korboulewsky *et al.*, 2002a; Martinez *et al.*, 2003a; Navas *et al.*, 1999). However, few have studied compost amendment effects on *in situ* planted tree seedlings, especially in fire-degraded Mediterranean areas.

The aim of this study was to determine over a three year period the effects of a sewage sludge co-composted with green wastes applied in a plantation. The aims were to (i) determine the effects of compost application on soil fertility; (ii) evaluate seedling survival, growth and nutrient status; (iii) evaluate trace metal contamination of soil and seedlings after amendment.

Material and methods

Site description

The experiment was carried out on a 3 ha site in the plateau of Arbois in the vicinity of Marseille (South Provence, France), at 240 m above sea level (5°18'6''E – 43°29'10''N in WSG-84 Geodetic system). The soil was a silty-clayey chalky rendzina with a low organic matter content (Table 1). It had a high percentage of stones (70 %) and a low average depth (24 cm). The last fire occurred in June 1995 and the site was colonized by a Mediterranean sclerophyllous vegetation, with a 70 % total cover before tree planting. *Quercus coccifera* L. and *Brachypodium retusum* Pers. were the two dominant species, implying a very fissured bedrock (Aubert, 1983). This natural vegetation belongs to the hoalm oak (*Quercus ilex* L.)

Table 1. Soil and compost characteristics, May 2001. Mean (SE), N=32. DM: dry matter; FM: fresh matter. Cations: available concentrations in soil and total concentrations in compost.

| Parameter | Soil | Compost | Calculated concentration increase shown for intermediate (D20) in soil fraction < 2mm (%) |
|---|----------------|------------|---|
| Density | 1.4 (0.1) | | |
| Granulometry | Clay (%) | 28.2 (0.7) | |
| | Silt (%) | 50.0 (0.8) | |
| | Sand (%) | 12.4 (1.2) | |
| Cation exchange capacity (<i>meq</i> .100g ⁻¹ DM) | 20.9 (0.8) | | |
| Humidity (% FM) | | 34 | |
| pH _{H2O} | 7.96 (0.01) | 7.70 | |
| C/N | 11.2 | 13.50 | |
| Organic matter (% DM) | 5.6 (0.2) | 39.00 | 33 |
| Total N (% DM) | 0.29 (0.02) | 0.69 | 11 |
| Total P (% DM) | 0.03 (0.003) | 0.59 | 94 |
| Extractable P (mg.kg ⁻¹ DM) | 7.2 (1.05) | 2515 | |
| Available / Total K (% DM) | 0.03 (0.0008) | 0.22 | |
| Available / Total Mg (% DM) | 0.012 (0.0003) | 0.17 | |
| Available / Total Ca (% DM) | 0.86 (0.014) | 5.30 | |
| Cd (mg.kg ⁻¹ DM) | 0.33 (0.03) | 0.77 | 11 |
| Cr (mg.kg ⁻¹ DM) | 50.7 (0.7) | 21.9 | 2 |
| Cu (mg.kg ⁻¹ DM) | 18.7 (0.5) | 122 | 31 |
| Hg (mg.kg ⁻¹ DM) | 0.06 (0.07) | 0.88 | 70 |
| Ni (mg.kg ⁻¹ DM) | 42.7 (0.3) | 14.7 | 2 |
| Pb (mg.kg ⁻¹ DM) | 35.2 (1.4) | 65 | 9 |
| Zn (mg.kg ⁻¹ DM) | 67.4 (1.5) | 266 | 19 |

The trace elements were extracted in aqua regia

succession series. During the two years of experimentation 2002 and 2003, the mean air temperatures were 14.1°C and 14.3°C respectively; the total annual rainfalls were 867 mm and 626 mm respectively. The site was strongly exposed to harsh North-West winds increasing natural drought. Before amendment, the soil had relatively high Cr, Ni and Pb concentrations (Table 1) (De Nicola *et al.*, 2003; Hernandez *et al.*, 2003), the area receiving significant acid atmospheric pollution (AIRMARAIX, 1999). Soil Cd concentration (0.33 mg.kg⁻¹ DM) was beyond the 0.1 mg.kg⁻¹ DM threshold reported to reduce agricultural crop yields by causing damage to root tissue (Brockway, 1983).

Compost

The six months maturity compost was produced by Biotechna (Ensuès, South Provence, France) and certified conform to the NF U 44-095 (2002) on composts made from materials of water treatment origin. This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 d at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in swathes. The swathes were mixed several times in the next 6 mo to promote organic matter humification. The final compost met the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals (Table 1).

Experimental design

The experimental design had two factor levels: compost rate and seedling species. Three blocks (1 ha) corresponding to compost rate were settled: control without compost (D0), 20 kg of fresh compost per seedling (D20) and 40 kg of fresh compost per seedling (D40). In each block, nine subplots (three per seedling species) of 30 individuals (three rows of ten seedlings) were randomly established.

The site preparation was as follow: first, three meters spacing plantation lines were cleared of above ground vegetation. Second, 1m²-holes were dug at a 4 m spacing, each one corresponding to one seedling. Third, a unique compost amendment took place in May 2001. The compost was mechanically mixed with the soil in each hole. The soil of the control block (D0) was mixed too. Considering a 400 seedling.ha⁻¹ density of plantation, the compost being

locally amended at each seedling stem, we globally spread 8 tons (D20) and 16 tons (D40) of fresh compost per hectare on the two amended blocks.

Seven months after the compost amendment (December 2001), 1-year seedlings of 3 species were planted: *Quercus ilex* L., *Pinus halepensis* Miller, and *Pinus pinea* L. At the stem of each seedling, a 40 x 40 cm cellulosic protection was laid on the ground to limit soil water loss and interspecific competition. Seedlings were watered once in August 2003 to avoid excessive drought mortality.

Soil sampling and analysis

Soil samples were collected before compost amendment (May 2001), at plantation time (December 2001), one year and a half (December 2002) and two years and a half after amendment (November 2003). Each soil sample was a mix of two samples collected at the stem of the two central seedlings in each subplot. The soil was collected inside a 40 cm diameter circle around the seedling stem, in the area covered by the cellulosic protection. All the accessible soil down to bedrock was collected. The soil samples were oven dried at 40°C to constant weight and sieved through a 2 mm mesh before analysis. Soil pH_{H2O} was measured using 10 g of dried ground soil added to 25 ml of distilled water (NF ISO 10390, AFNOR, 1999; PHM 240 Radius méter). Three-fraction granulometry was determined gravimetrically (NF X 31-107, AFNOR, 1999). Cation exchange capacity (CEC) was measured by soil percolation with an ammonium acetate solution (NF X 31-130, AFNOR, 1999) and organic C was determined using sulphuric-chromic oxidation and spectrophotometry (NF ISO 14235, AFNOR, 1999; Cary 50 VARIAN). Soil organic matter concentration was calculated as 1,72 x organic carbon (C). Available potassium (K), calcium (Ca) and magnesium (Mg) were 1M NH₄Ac extracted and analysed by atomic absorption spectrophotometry (NF X 31-108, AFNOR, 1999; FS220 VARIAN). Total nitrogen (N) was determined by dry combustion and thermic conductimetry (ISO 13878, AFNOR, 1999; FP 428 LECO). Total phosphorus (P) and iron (Fe) were measured according to NF EN ISO 11885 (VARIAN VISTA Radial), and extractable P was extracted with sodium hydrogenocarbonate solution determined by spectrophotometry (Olsen *et al.*, 1954) (NF EN ISO 11263, AFNOR, 1999; Cary 50 VARIAN). Cu and Zn available forms were extracted using EDTA and the elements in the solution were assessed by atomic absorption spectrophotometry (NF X 31-120, AFNOR, 1999; VARIAN VISTA Radial). To measure total concentrations of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and

zinc (Zn), soil samples were digested in *aqua regia* and analysed using plasma emission spectrophotometry (NF EN ISO 11466, AFNOR, 1999; VARIAN VISTA Axial). Mercury (Hg) was extracted according to NF X31-151 and determined using plasma emission spectrophotometry (VARIAN VISTA Axial).

Seedling survival, growth and chemical analysis

Seedling survival and growth were measured one year (December 2002) and two years after planting (November 2003). The annual length growth was measured as the difference between maximal seedling lengths at one year interval (December 2001 - December 2002 and December 2002 - November 2003). Likewise, the annual radial growth was calculated as the difference between seedling basal stem diameters at one year interval. Both length and diameter were estimated on five seedlings per subplot of 30 seedlings, these five seedlings being fixed for all the experiment duration. Three seedlings per subplot were chosen randomly in December 2002 and November 2003. Their leaves and needles were collected by species for N, P, cations and trace metal analysis. The foliar material was washed with demineralized water, oven dried at 40°C and 2 mm mesh sieved in a trace metal free grinder (made with tungstene carbure, FOSS TECATOR Sample Mill 1093 Cyclotec). For N, K, Ca and Mg analysis, 250 mg of sample were digested one night in 5 ml H₂SO₄ (96%) and 5 ml H₂O₂ (35%), then heated at 400°C during 3 hours (Bioblock Scientific Digestion Unit 10401). Then, the solutions were diluted 500 times by adding ultrapure water (Millipore Symplicity 185), filtered at 0.45µm and analysed by ion chromatography (Dionex DX120) (method adapted from Masson and Andrieu, 1996). The eluent used was 26 mmol.L⁻¹ methan sulfonic acid. Total concentrations of the other elements, P, Cd, Cr, Cu, Ni, Pb and Zn, were measured after digestion in *aqua regia* using atomic absorption spectrometry.

Statistical analysis

When the conditions of normality and variance homogeneity (verified by Shapiro-Wilks and Bartlett tests respectively) were checked, data were interpreted using analysis of variance and means were compared by using least significant different values (Tukey's test, 0.05 significance level) (Zar, 1984). In case of non homogeneity of variance, the Kruskal-Wallis test was used followed by the Newman-Student-Keuls test. Seedling survival was compared

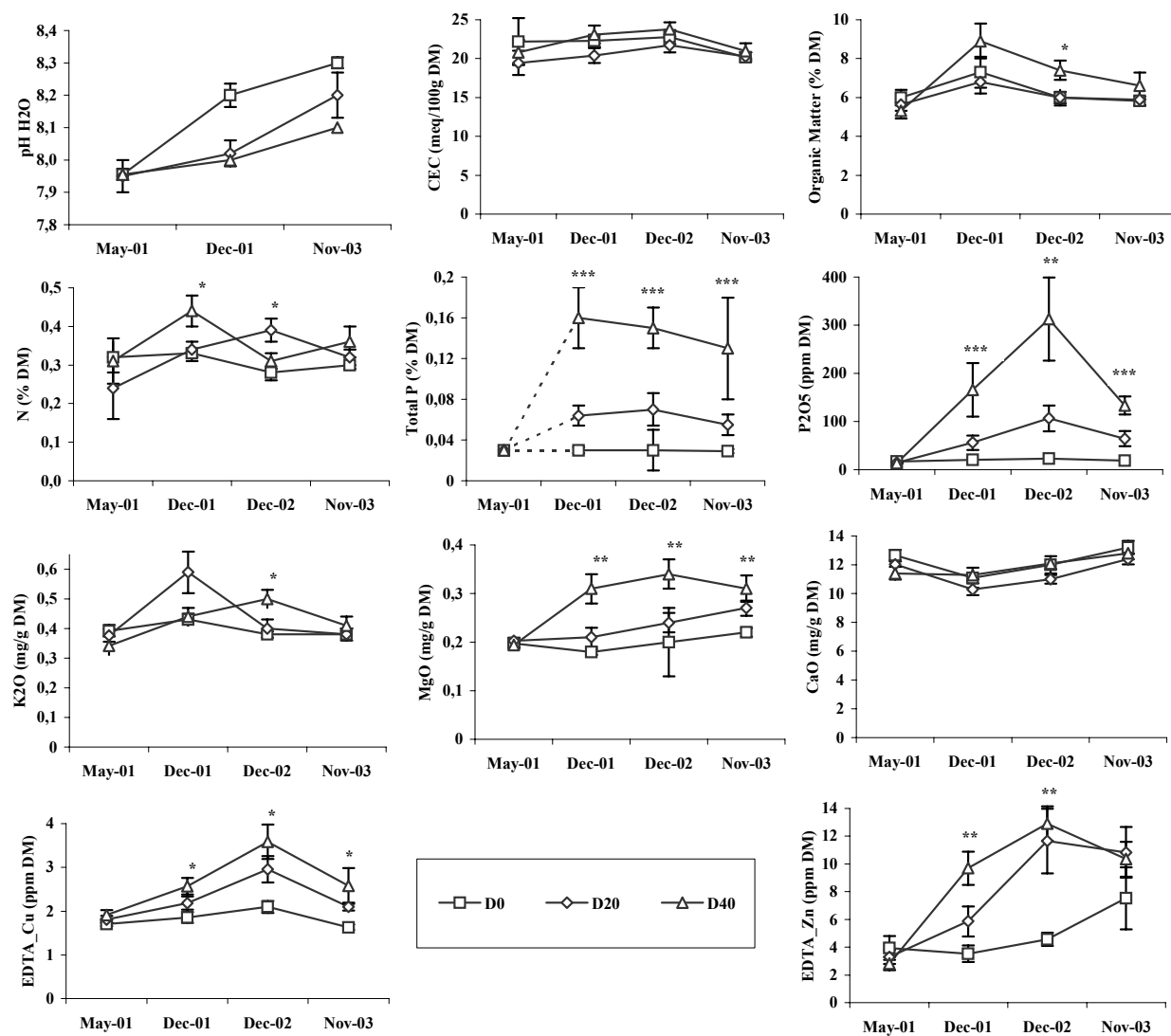


Figure 1. Soil properties before amendment (May 2001), seven month after amendment (at plantation time in December 2001), one year (December 2002) and two years (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. Mean \pm SE, $N \geq 9$. * $0.05 < p < 0.01$; ** $0.01 < p < 0.001$; *** $p < 0.001$.

with Chi^2 (0.01 significance level). The software Statgraphics plus (version 2.1: Statistical Graphics Corporation, © Copyright 1994-1996) was used.

Results

Soil agronomic properties

The amendment decreased pH slightly, especially on D40 amended blocks 7 mo and 2.5 yr after amendment. However, compost amendment had no significant effect on soil cationic exchange capacity.

Compost significantly increased the nutritional potentialities of the soil (Figure 1). Indeed, the soil organic matter concentration increased in D40 block after amendment, but the effect declined over time. In addition, total N concentration increased in amended blocks 7 mo (for D40) and 1.5 yr (for D20) after amendment. But as with OM, this effect disappeared 2.5 yr after amendment.

Soil total and extractable P concentration also were significantly increased by the compost. In D40 block, soil total P concentration increased up to 5 times compared to control (7 mo and 1.5 year after amendment). In addition and unlike total soil N, this effect remained until the end of the experiment. Indeed, soil total P concentration in D40 block was still four times greater 2.5 yr after amendment. Similarly, the soil extractable P concentration was remarkable in amended blocks, especially in D40 block. In this block, soil extractable P concentration reached 313.1 ppm DM 1.5 yr after amendment, which is more than 13 times higher than in the control.

Available soil cation concentrations were affected differently. Available Mg concentration was significantly increased in D40 treatment to the end of the study period, but Mg concentrations in D20 were not different from the control. The compost increased available K concentration after amendment. This effect was significant in D40 1.5 yr after treatment, then disappeared by 2.5 yr.

Soil trace metals

Soil total Cu and Zn concentrations increased in amended blocks both for D20 and D40 blocks at 7 mo and 1.5 yr after amendment and then decreased to the values obtained in the control soil by 2.5 yr. The increase reached 55 % in D40 and 25 % in D20 for soil total Cu,

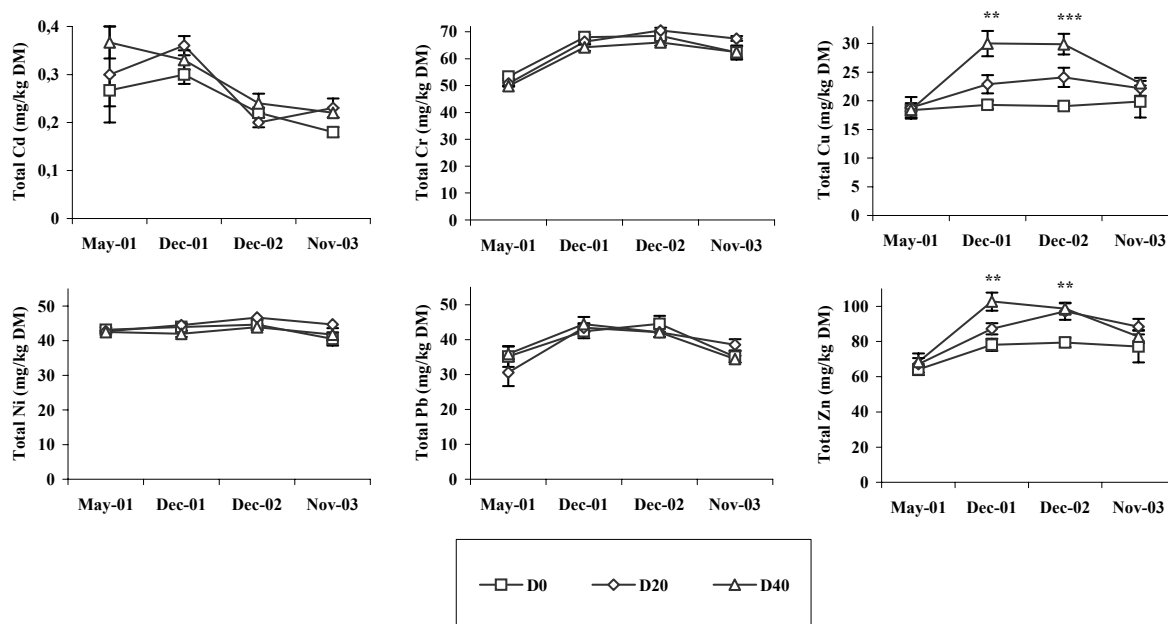


Figure 2. Soil trace metal concentrations before amendment (May 2001), seven month after amendment (at plantation time in December 2001), one year (December 2002) and two years (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. Mean \pm SE, $N \geq 9$. * $0.05 < p < 0.01$; ** $0.01 < p < 0.001$; *** $p < 0.001$.

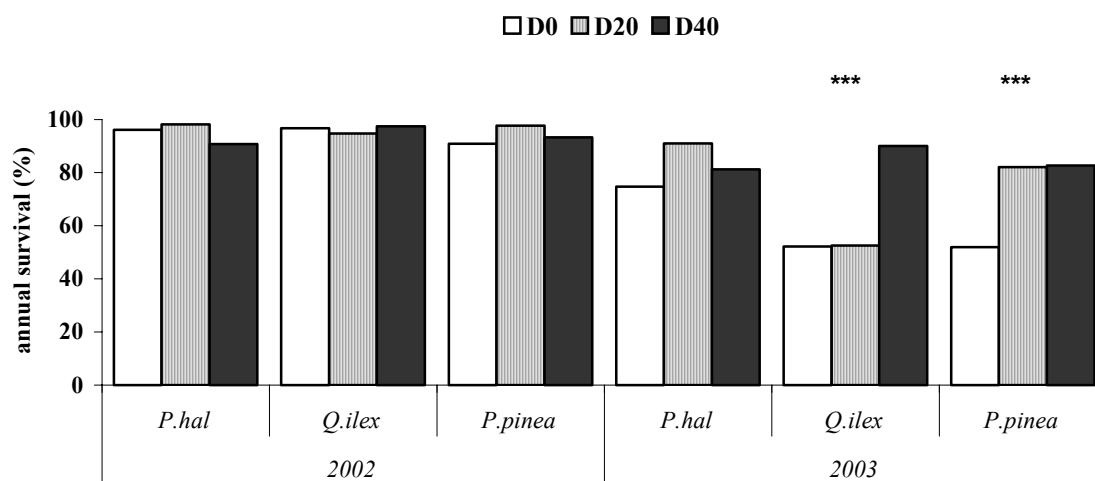


Figure 3. Annual survival (%) one year (December 2002) and two years (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. ***significant at the 0.01 level.

and 25 % in D40 and 12 % in D20 for soil total Zn. This compost effect was significant in D40 treatment for both elements, and in D20 treatment only for Zn 1.5 yr after amendment. Copper and Zinc available concentrations increased significantly in D40 treated soil in comparison with control (Figure 2). This effect was maximal 1.5 yr after amendment for both elements. It remained 2.5 yr after amendment for Cu, while it disappeared for Zn. Furthermore, the increase was also observed in D20 treatment for soil available Zn concentration.

Soil concentrations of the other total trace elements (Cd, Cr, Ni and Pb) were not significantly affected by the amendment.

Seedling responses to compost amendment

Survival rates were not affected by compost amendment in 2002, the first year after plantation, while they were improved for *Quercus ilex* and *Pinus pinea* in 2003, the second year after planting (Figure 3). Indeed, in 2003, survival was significantly higher in D40 for *Quercus ilex* (+40%), and in both compost doses for *Pinus pinea* (+30%) compared to control. For all species and treatments, survival rates in 2003 were lower than in 2002.

Annual growth rates (length and radial growth) changed between blocks, years, and species (Figure 4). For all species and both years, the compost amendment increased length growth by 2 to 3 times and doubled radial growth in amended blocks compared to control (length growth increased only slightly in D20 block in 2003). Similarly to survival rates, annual growth rates were lower in 2003 in comparison with 2002.

The seedling species were differently affected by the compost amendment only in 2002 (two way ANOVA, species x compost rate, $p=0.031$) (Figure 5). Indeed, *Pinus pinea* length growth increased proportionally to compost rate (33.5, 54.4 and 72.7 % respectively for D0, D20 and D40). On the contrary, *Pinus halepensis*, and to a lesser extent *Quercus ilex*, had a maximal length growth in D20 block. Indeed, for those 2 species, length growth in D40 block (52.5 and 33.5 % respectively for *P.halepensis* and *Q.ilex*) was intermediate between D20 block (100.5 and 46.5 %) and control (18 and 24.8 %).

The foliar N and P concentrations of seedlings were significantly higher in amended treatments than in control for both 2002 and 2003 (Table 2). The foliar P concentration significantly increased with the rate of compost in 2002 (D20 significantly different from

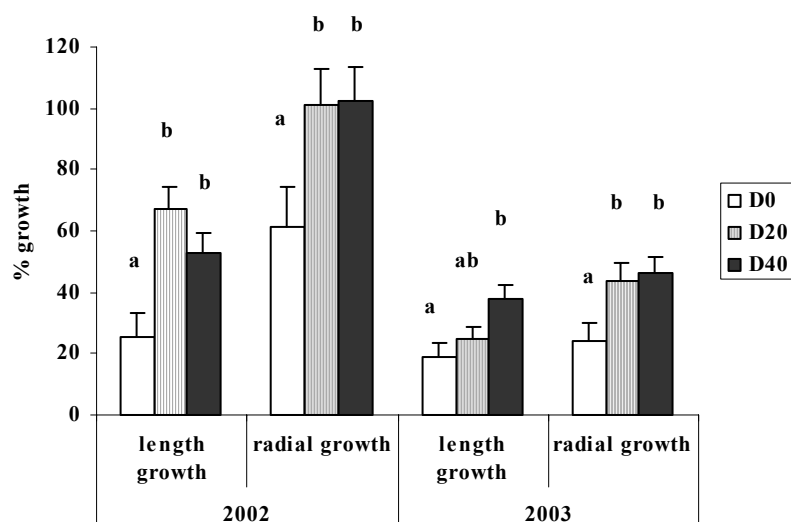


Figure 4. Mean annual length and radial growth (%) one year (December 2002) and two years (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. The three seedling species are considered all together. Bars denote SE, $N \geq 9$. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter.

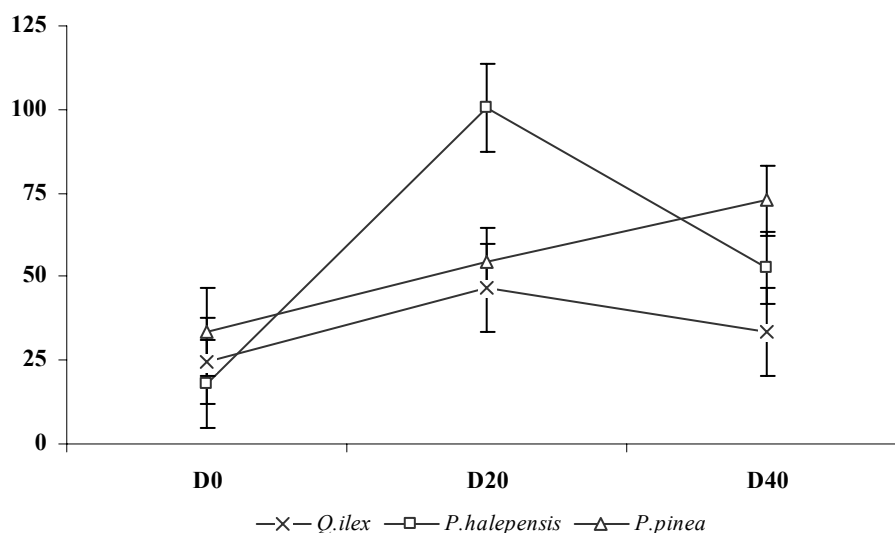


Figure 5. Comparison between the three seedling species length growth (%) one year (December 2002) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. Mean \pm SE, $N=4$.

D40) and in 2003. Nitrogen foliar concentrations were similar in D20 and D40 treatments for both years, and lower in 2003 than in 2002. Compost amendment increased significantly foliar K concentration in 2002 for both compost rates. This effect did not arise in 2003. Compost had no effect on foliar Mg and Ca concentrations.

Seedlings trace metal concentrations

Compost had no significant effect on foliar trace metal concentrations (Table 3). However, in 2002, foliar concentration of Cr, Cu, Ni and Zn averaged higher in compost amended blocks than in control. Moreover, as for N, foliar trace metal concentrations were lower in 2003 than in 2002. Differences, however, were not statistically significant.

Discussion

Effects on soil

Compost amendment on a degraded calcareous rendzina improved soil quality by increasing total N, P, and available Mg concentrations, and slightly available K and OM. These positive effects on soil were maximal for the highest rate of compost (40 kg.m⁻²). Indeed, sewage sludge is a rich source of macronutrients and organic C, because it concentrates salts from water purification, these salts being fixed in microflora. Thus, improvement of soil fertility after compost amendment has been widely observed (Guerrero *et al.*, 2001; Korboulewsky *et al.*, 2002b; Martinez *et al.*, 2003a) and is the reason of its use in agriculture. However, these improvements effects disappeared 2.5 yr after amendment for N, OM and K. Likewise, Martinez *et al.* (2003a) and Guerrero *et al.* (2001) found that compost effects on soil decreased over time, as its OM mineralized. In our experiment, the disappearing effect of N enrichment can have two combined explanations. On one hand, N is probably the limiting nutrient in our ecosystem, as with most terrestrial ecosystems (Archibold, 1995; Davey and Wollum, 1984; Thirukkumaran and Parkinson, 2002), and may have been highly absorbed by the seedlings, as the foliar concentrations and the growth rates suggest. On the other hand, part of N could have been leached in the soil (NO₃⁻ form, Sands, 1984) especially that the highly fractured bedrock (Aubert, 1983) favours its transport to groundwater. Thus, N may have been progressively removed from the soil by these two ways and 2.5 years after amendment its total concentration had met the control one. On the contrary, as P and Mg are

Table 2. Nutrient concentrations in the three seedling species leaves or needles one year (December 2002) and two years (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. Mean (SE), $N \geq 9$. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter.

| | | N | P | K | Mg | Ca |
|------|-----|---------------------------|---------------------------|--------------------------|---------------------------|--------------------------|
| | | (% DM) | (% DM) | (% DM) | (% DM) | (% DM) |
| 2002 | D0 | 1,28 (0,08) ^a | 0,11 (0,008) ^a | 0,59 (0,05) ^a | 0,11 (0,004) ^a | 0,54 (0,05) ^a |
| | D20 | 1,38 (0,06) ^{ab} | 0,17 (0,019) ^b | 0,65 (0,06) ^b | 0,12 (0,003) ^a | 0,56 (0,05) ^a |
| | D40 | 1,53 (0,07) ^b | 0,19 (0,020) ^c | 0,66 (0,06) ^b | 0,11 (0,003) ^a | 0,51 (0,04) ^a |
| 2003 | D0 | 1,15 (0,05) ^a | 0,11 (0,007) ^a | 0,45 (0,04) ^a | 0,1 (0,006) ^a | 0,49 (0,02) ^a |
| | D20 | 1,37 (0,05) ^b | 0,15 (0,016) ^b | 0,5 (0,05) ^a | 0,11 (0,006) ^a | 0,53 (0,03) ^a |
| | D40 | 1,41 (0,06) ^b | 0,14 (0,015) ^b | 0,5 (0,06) ^a | 0,11 (0,005) ^a | 0,48 (0,04) ^a |

Table 3. Trace metals concentrations (mg/kg DM) in the three seedling species leaves or needles one year (December 2002) and two years (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) blocks. Mean (SE), $N \geq 9$. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter.

| Compost rate | Cd | Cr | Cu | Ni | Pb | Zn | |
|--------------|--------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| | (mg.kg ⁻¹ DM) | (mg.kg ⁻¹ DM) | (mg.kg ⁻¹ DM) | (mg.kg ⁻¹ DM) | (mg.kg ⁻¹ DM) | (mg.kg ⁻¹ DM) | |
| 2002 | D0 | 0.05 (0.009) ^a | 1.5 (0.5) ^a | 5.7 (0.9) ^a | 0.34 (0.18) ^a | 0.83 (0.13) ^a | 75 (4.6) ^a |
| | D20 | 0.06 (0.005) ^a | 1.95 (0.7) ^a | 5.9 (0.6) ^a | 0.54 (0.34) ^a | 0.73 (0.11) ^a | 91 (5.1) ^a |
| | D40 | 0.04 (0.013) ^a | 2.12 (0.6) ^a | 6.6 (0.6) ^a | 0.45 (0.28) ^a | 0.78 (0.1) ^a | 93 (12.7) ^a |
| 2003 | D0 | <0.01 | 0.59 (0.09) ^a | 5.5 (0.2) ^a | 0.38 (0.04) ^a | 0.79 (0.14) ^a | 94 (8.9) ^a |
| | D20 | <0.01 | 0.69 (0.09) ^a | 5.9 (0.2) ^a | 0.38 (0.03) ^a | 0.76 (0.14) ^a | 100 (10.4) ^a |
| | D40 | <0.01 | 0.58 (0.08) ^a | 5.7 (0.4) ^a | 0.36 (0.04) ^a | 0.61 (0.08) ^a | 91 (8.6) ^a |

brought in much higher amounts, their concentrations remained higher until the end of the experiment compared to control. Indeed, compost highly enriched the experimental soil in P and Mg in comparison with N (Table 1).

Compost amendment induced a remarkable increase of extractable Phosphorus in amended soil. Thus, in D40 amended soil extractable P concentration reached 13 times the value measured in control 1.5 yr after amendment. Indeed, the sewage sludge entering compost composition comes from a sewage plant that applies a dephosphatation process to sewage. Thus P is concentrated in the sludge, and the latter greatly enriches the compost in P. Likewise, [Martinez et al. \(2003a\)](#) reported a ten fold increase of extractable P in biosolids amended soil 1 yr after amendment. Similarly to total P, this extractable P increase on D40 amended block remained significant 2.5 years after amendment. Although P is not particularly mobile, its buildup exceeds plant uptake and could lead to serious water pollution problems and limits the rates of compost which can be used.

Soil CEC seemed to increase in amended treatments, but not significantly. Indeed, [Baize \(1988\)](#) reported that silt and particularly clay have a high cation adsorption capacity. Thus, the silty-clayey soil should have a very elevated natural CEC, besides which the effect of compost OM input on CEC may be negligible.

As compost pH is lower than the calcareous soil pH, the amendment slightly decreased the amended soil pH. However, the slight difference between soil and compost pH and the large sampling area (1 ha per block) might have hidden the effect of amendment and the pH decrease was not significant, though Figure 1 shows a clear tendency.

Effects on seedlings

Compost amendment improved the survival of the two seedling species (*Quercus ilex* and *Pinus pinea*) under drought conditions in 2003, whereas it had no effect on this parameter the first year after plantation in 2002. On the contrary, *Pinus halepensis* survival was not significantly affected by compost amendment. *Quercus ilex* and *Pinus pinea* survival increase might be related to their better nutritional status (N, P, K) on compost amended blocks, especially to higher foliar K concentrations. Indeed, [Welander and Ottosson \(2000\)](#) reported that transpiration rates of oak seedlings decreased with nutrient strength. Moreover, high levels of foliar K are associated with reduced transpiration rates in several tree species, due to the regulatory effect of this ion on the stomatal movement ([Van Den Driessche, 1984](#)) and therefore improve seedlings water saving in case of drought. Moreover, [Khanna and Ulrich](#)

(1984) reported that K deficiencies may particularly occur in plantations on calcareous soils. Thus, the additional K brought by compost should be of greater use and a drought protection for seedlings.

Foliar nutrient levels in seedlings grown on compost amended blocks were higher than seedlings grown on control for N, P and K. [Moreno *et al.* \(1996\)](#) reported same results for barley grown on calcareous soil amended with sewage sludge compost.

Compost amendment had no significant effect on Mg and Ca concentrations in foliar parts of seedlings, although compost amendment induced significant and durable available Mg increase in soil. This might have been due to the formation of humates and fulvates between the organic matter of the compost and this element, so that it is retained in a form unavailable to plants ([Moreno *et al.*, 1996](#)).

The increased growth of seedlings in compost amended blocks may be linked to their better nutritional status (NPK) ([Martin-Prével *et al.*, 1984](#)). Indeed, compost increased the length growth of the three seedling species by 2.0 and radial growth by 1.7 for both years and both compost rates (D20 and D40). As foliar N concentration increased in seedlings grown on compost amended blocks, this nutrient may be highly responsible for growth increase, and P to a lesser extent ([McKay and Moffat, 2001](#)).

[McKay and Moffat \(2001\)](#) reported similar results for Sitka spruce after sewage sludge application at planting. Likewise, [Brockway \(1983\)](#) and [Dutch and Wolstenholme \(1994\)](#) found sewage sludge amendment to increase the growth of Sitka spruce and red pine.

The three species did not respond similarly to compost amendment for growth. The D40 compost rate induced maximal length growth in 2002 for *Pinus pinea* seedlings, even though it was D20 compost rate for *Pinus halepensis* and *Quercus ilex* seedlings. As compost induced very elevated concentrations of extractable P in D40 amended block, and as plant growth reductions have been reported when soil P is in excess (tomatoes, [Bingham, 1966](#)), excessive extractable P may have reduced *Pinus halepensis*, and *Quercus ilex*, in blocks amended at highest rate. Thus, in *Pinus halepensis* and *Quercus ilex* plantations, P should limit compost application to a rate below 40 kg.m⁻². However, as compost improvement of *Quercus ilex* survival was only significant for D40 (+ 40 %), this conclusion has to be put in perspective for this species.

For all species and treatments, the seedling survival, growth and foliar nutrient concentrations were lower in 2003 than in 2002. Indeed, seedlings had to cope with severe drought the summer 2003. As drought decreases soil water content, and as soil nutrients are available to plants in soluble forms, drought decreases the nutrient absorption by plants, leading to worse foliar nutritional status. Moreover, drought, by increasing water deficits, is responsible for survival and growth rates decreasing.

Trace metal concentrations

Although compost is an extra source of heavy metals, we did not find a significant increase of total Cr, Cd, Ni and Pb in the soil during the experiment (2.5 yr). Indeed, the compost used had lower concentrations in Cr, Ni and Pb than the soil and was weakly contaminated in Cd (Table1). The high pH of the calcareous soil, which favours the immobilization of most heavy metals in the soil (Martinez *et al.*, 2003a), may have protected seedlings from their absorption. Thus, no trace metal contamination induced by compost appeared in foliar parts of the seedlings after amendment. Moreover, the Cd, Cr, Pb and Ni concentrations were far below toxic limits in sensitive plants species (Kabata-Pendias and Pendias, 1992). However, these elements remain for very long periods of time in the soils (Dai *et al.*, 2004) and further changes in physicochemical soil conditions could induce some remobilization of these toxic elements and increase their disponibility for seedlings (Planquart *et al.*, 1999).

Among trace metals, only total and available Cu and Zn soil concentrations increased significantly after compost amendment at D40 rate. But it had no significant consequence on seedling foliar concentrations in these metals. In calcareous soil, Ca^{2+} as the major ion reduces the plant absorption of other polyvalent cations (competitive ion effect). Thus, calcium may have prevented Cu and Zn from being absorbed despite their increased concentrations in the soil. In addition, to assess seedling Cu absorption, foliar concentrations may not be good indicators because of preferential root accumulation and restricted transport to foliage (Van Den Driessche, 1984; Wisniewski and Dickinson, 2003). Furthermore, phosphorus, present at high concentrations in amended soil, might have complexed with Cu and Zn, depleting their absorption by seedlings (Kabata-Pendias and Pendias, 1992; Khanna and Ulrich, 1984).

The soil available Zn and total Cu and Zn enrichment effect of compost had disappeared 2.5 yr after amendment. Since no significant foliar absorption of Cu and Zn was found on amended blocks, these elements might have been deep leached through the highly fractured bedrock, explaining total concentrations decrease. Moreover, the progressive degradation of OM reduced the number of site fixation for cations, inducing an increase in their leachability, especially for Cu (Moreno *et al.*, 1996). Thus, it is very likely that available Cu concentration will rapidly decrease to control concentration, even if it remained significantly higher in D40 block soil compared to control at the end of the experimentation.

Conclusion

Compost improved *Quercus ilex* and *Pinus pinea* survival under drought, as well as the 3 species NPK nutrition and growth. Furthermore, it did not induce trace metal foliar contamination. In consequence, the use of a mature and low heavy metal contaminated compost may be an interesting practice in Mediterranean region to restore nutritional potentialities on degraded soils, and create better conditions for tree installation.

The D40 dose globally led to higher soil fertility and seedling growth than D20. But it seemed that the extractable P concentration in D40 amended soil limited *Pinus halepensis* and *Quercus ilex* length growth in 2002, whereas their growth was improved in D20 amended block. Thus, in *Pinus halepensis* and *Quercus ilex* plantations, P should limit compost application to a rate below 40 kg.m⁻², even if this rate improved *Quercus ilex* survival. As tree species do not react the same way to compost amendment, it would be of great interest to study which rate is the most beneficial to each species before setting any plantation.

In addition, soil extractable P could be leached and highly increased concentration in amended soils might induce ground water pollution. Moreover, as total and available Cu and Zn concentrations highly increased after amendment, they could increase site pollution. Hence, P is the limiting factor for this sewage sludge and green wastes compost use, and in a lesser extent Cu and Zn. In consequence, to prevent pollution, amendment composition and rates, vegetation cover and site hydrographic characteristics must be well studied and defined before experimental setting.

Acknowledgements

This research has been commissioned by the Conseil Général des Bouches-du-Rhône (France), and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the French Rhône-Méditerranée-Corse Water Agency.

Résultats

L'apport de compost a amélioré la fertilité du sol en augmentant les teneurs en matière organique et en macroéléments. Cet effet est maximal pour la dose D40. La durabilité des effets diffère selon l'élément considéré, en relation avec la quantité totale apportée par le compost. Deux ans après épandage, les concentrations en N et en K du sol ne se différencient plus de celles du sol témoin, alors que celles du P et du Mg restent plus élevées. Les teneurs en P échangeable du sol sont multipliées par 13 après épandage sur D40, ce qui accroît les risques de pollution des eaux de nappe et de surface.

La présence de compost améliore les taux de survie de *Q. ilex* (D40) et de *Pinus pinea* (D20 et D40) en période de sécheresse (2003), alors qu'il n'a pas d'effet sur la survie de *P. halepensis*, qui reste élevée malgré la sécheresse. Cet effet bénéfique est probablement dû à l'amélioration du statut nutritionnel des plants. En effet, les plants des parcelles amendées présentent des teneurs foliaires en N, P et K plus élevées par rapport au témoin. Il est à remarquer que l'augmentation importante de la concentration en Mg du sol après amendement n'induit pas d'augmentation significative de cet élément dans les feuilles.

La croissance des plants est stimulée par l'apport de compost, mais l'effet diffère en fonction de l'espèce. La stimulation est maximale pour *P. halepensis* et minimale pour *Q. ilex*. La croissance sur D40 est plus faible que sur D20 pour ces deux espèces, ce qui laisse penser que les fortes teneurs en P sur D40 ont dépassé le seuil optimal. Par contre, la croissance de *P. pinea* est proportionnelle à la dose de compost.

L'apport de compost n'enrichit pas significativement le sol en Cr, Cd, Ni et Pb au cours des deux ans et demi qui suivent l'épandage. De plus, aucune contamination des plants par ces quatre éléments n'est mise en évidence.

Par contre, les teneurs en Cu et Zn du sol augmentent après épandage sur D40. Cependant, ces métaux ne sont pas absorbés par les plants, probablement du fait de leur immobilisation dans le sol calcaire. Par contre, il est possible que l'augmentation de ces métaux dans le sol a induit leur lessivage vers l'eau des nappes ou de surface.

Chapitre 2

LE COMPOST PEUT-IL FAVORISER L'INSTALLATION DE *QUERCUS PUBESCENS* DANS DES SOLS DEGRADEES EN REGION MEDITERRANEENNE ?

Can compost improve *Quercus pubescens* Willd installation in degraded sloping soil in the Mediterranean area ?

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Article envoyé le 19 juillet 2004 à Bioresource Technology

Abstract

Since sewage sludge dumping has been banned in the EEC, there is renewed interest in studying new ways of recycling it. There are in the Mediterranean area a lot of degraded burnt lands to reforest and organic amendment could improve soil quality and so favour tree installation.

A frequently burnt calcareous site in Provence (France) characterized by steeply sloping soil, drought and low organic matter content was chosen. We planted one-year tree-seedlings of *Quercus pubescens* Willd. Three rates of co-composted sewage sludge and greenwastes were incorporated into the soil at the seedling stalk: 40 kg, 20 kg and a control. Changes in soil properties were monitored for 3 years (2001-2003) and tree survival, growth and nutrient status for 2 years (2002-2003).

Compost improved soil fertility at both rates by increasing organic matter (OM), cation exchange capacity (CEC), total N, exchangeable P, K, Mg and B concentrations. However,

the compost had no effect on either seedling survival or growth, but increased foliar P and B concentrations at the D40 rate. Moreover, no foliar contamination of seedlings by trace metals occurred, although amendment increased total Cd, exchangeable Cu and Zn concentrations in amended soil.

Keywords: *Quercus pubescens*, compost, reforestation, Mediterranean area.

Introduction

Mediterranean areas undergo recurrent fires, which decrease soil fertility (De Luis *et al.*, 2001). Fire consumes organic matter (OM) and its nutrient content is either volatilized or deposited in the ash (Grogan *et al.*, 2000), thus giving rise to leaching. Moreover, by removing vegetation cover and soil OM, fire decreases the resistance of soil aggregates and increases erosion, especially on steep slopes (Giovannini *et al.*, 2001). There, the continual removal of weathered rock material has resulted in rather thin, infertile soils which are often deficient in N and P (Archibold, 1995), and have low water availability (Le Houerou, 1973). Furthermore, these soils are colonized by a garrigue vegetation composed of drought tolerant and highly inflammable plants (Le Houerou, 1973).

Quercus pubescens Willd, as a deciduous oak species, is less inflammable than garrigue vegetation (Quézel and Médail, 2003). Thus, its rehabilitation on degraded soils could be a way to decrease fire risks. *Quercus pubescens* is not affected by pH but prefers wetter areas with deeper soils or north-facing slopes (Damesin *et al.*, 1998). In Southern France, both phytosociological and paleobotanical studies have shown that, as living conditions became worse, downy oak (*Quercus pubescens* Willd) was progressively replaced by *Quercus ilex* (Planchais *et al.*, 1977; Vernet, 1973).

Organic amendment could create better conditions for the installation of *Quercus pubescens*. Querejeta *et al.* (2001) reported that materials rich in C compounds easily decomposable by soil microorganisms can raise fertility levels, stimulate soil aggregate formation and stabilization and thus enhance hydraulic permeability and increase water holding capacity.

Sewage sludge can improve the soil's physical and chemical properties (Caravaca *et al.*, 2002; McKay and Moffat, 2001), since it is a source of OM and plant nutrients (Martinez *et al.*, 2003a; Brockway, 1983). But if sewage sludge recycling may seem to be an attractive way to enhance soil potentialities and an alternative to landfill disposal, it nevertheless presents potential environmental risks. Sewage sludge amendment increases the soil's low-molecular soluble organic matter content and soluble salts (*e.g.* carbonates, phosphates from water

purification and newly-formed biological breakdown products such as NH_4^+ and HCO_3^-) (Agassi *et al.*, 1998), which on slopes can be leached to surface water (Martinez *et al.*, 2003b) and induce eutrophication. Moreover, its use can induce heavy metal and organic contaminant accumulation in soils (Brockway, 1983), which can lead to soil and water pollution.

Composting is a biological process of aerobic decomposition, which degrades labile organic matter to carbon dioxide, water vapour, ammonia and inorganic nutrients to obtain a stable organic material (compost) containing humic-like substances (Senesi, 1989). It may stabilize the organic matter content of sewage sludge and in this way decrease the risks of heavy metal and salt leaching (Planquart *et al.*, 1999; Garcia *et al.*, 1990). Moreover, the use of other organic wastes with large C/N ratios (such as green wastes) in mixture with biosolids can reduce the rate of nitrogen mineralization and then decrease the risk of leaching (McKay and Moffat, 2001): the release of plant available N from organic amendment is controlled by the balance of N immobilization and mineralization, which in turn, is controlled to a large extent by the C/N ratio of the decomposing organic material (Cambardella *et al.*, 2003).

Thus, it has been demonstrated that compost amendment can improve the soil's biological properties and consequently speed plant installation (Korboulewsky *et al.*, 2002a; Martinez *et al.*, 2003a; Dutch and Wolstenholme, 1994; Brockway, 1983; Navas *et al.*, 1999). But no study has yet assessed compost's ability to improve the installation of *Quercus pubescens* (? reforestation by *Quercus pubescens* / to facilitate planting ..) in the Mediterranean region.

Our aims were to (i) determine the effects of compost on soil properties, (ii) assess *Quercus pubescens* seedling survival, growth and nutrition, and (iii) study possible contamination of the soil and *Quercus pubescens* by compost trace metals.

Material and methods

Site description

The experiment was carried out on a 600 m² site, in the vicinity of Marseilles (Southern Provence, France), at 240 m above sea level (5°18'6''E – 43°29'10''N in WSG-84 Geodetic system). The soil was a silty-clayey chalky rendzina with low organic matter content (Table 1). This shallow soil (24 cm depth) had a high percentage of stones (70 %). The slope was 22° on a north-west exposure. The last fire occurred in June 1995 and the post-fire vegetation was Mediterranean sclerophyllous shrubland, with 70 % total cover. The natural vegetation belongs to the holm oak (*Quercus ilex* L.) succession series. However, below the

Table 1. Soil and compost characteristics, May 2001. Mean (SE), N=22. DM: dry matter; FM: fresh matter. Cations: exchangeable concentrations in soil and total concentrations in compost.

| Parameter | unit | Soil | Compost | D20 calculated concentration increase in soil fraction < 2mm (%) |
|--------------------------|---------------------------|--------------|------------|--|
| Density | | 1.4 (0.09) | | |
| Granulometry | clay | % | 29.4 (0.5) | |
| | silt | % | 46.6 (3.3) | |
| | sand | % | 10.1 (1.1) | |
| Cation exchange capacity | meq.100g ⁻¹ DM | 21.2 (1.0) | | |
| Humidity | % FM | | 34 | |
| pH _{H2O} | | 8 (0.02) | 7.7 | |
| C/N | | 11.7 | 13.5 | |
| Organic matter | % DM | 6.9 (0.5) | 39 | 27 |
| Total N | % DM | 0.31 (0.02) | 0.69 | 11 |
| Total P | % DM | 0.03 (0.003) | 0.59 | 94 |
| Exchangeable P | ppm DM | 19.6 (1.4) | 2515 | |
| Exchangeable / Total K | % DM | 0.04 (0.003) | 0.22 | |
| Exchangeable / Total Mg | % DM | 0.02 (0.001) | 0.17 | |
| Exchangeable / Total Ca | % DM | 1.36 (0.2) | 5.3 | |
| Exchangeable Cu | ppm DM | 1.34 (0.05) | | |
| Exchangeable Zn | ppm DM | 3.15 (0.4) | | |
| Exchangeable B / Total B | ppm DM | 1.52 (0.09) | 24.35 | |
| Cd | mg.kg ⁻¹ DM | 0.3 (0.03) | 0.77 | 12 |
| Cr | mg.kg ⁻¹ DM | 54.5 (2.6) | 21.9 | 2 |
| Cu | mg.kg ⁻¹ DM | 16.9 (0.7) | 122 | 34 |
| Hg | mg.kg ⁻¹ DM | 0.07 (0.01) | 0.88 | 60 |
| Ni | mg.kg ⁻¹ DM | 41.5 (1.0) | 14.7 | 2 |
| Pb | mg.kg ⁻¹ DM | 39.3 (4.3) | 65 | 8 |
| Zn | mg.kg ⁻¹ DM | 72.8 (3.9) | 266 | 17 |

experimental plots, some natural regeneration of *Quercus pubescens* occurs. During the two years of experimentation 2002 and 2003, the mean air temperatures were 14.1°C and 14.3°C respectively; the total annual rainfalls were respectively 867 mm and 626 mm. The site was strongly exposed to harsh north-west winds increasing natural drought conditions.

Experimental design

Three 200-m² plots corresponded respectively to 3 amendment rates : 40 kg (D40) and 20 kg (D20) of fresh compost per seedling and a control without compost (D0). For a 400 seedling.ha⁻¹ density of plantation, the compost being locally amended at each seedling stalk, we spread overall 8 tons (D20) and 16 tons (D40) of fresh compost per hectare on the two amended plots.

Amended plots were below the control plot to avoid water run-off contamination. In each plot, ten rows (4 m spacing) of five seedlings were planted. The site preparation was light. The seedlings future emplacements were cleared of above ground vegetation and then 1m²-holes were dug (Photo 1).

A single compost amendment was done in May 2001. The compost was mechanically mixed with the soil in each hole. The soil of the control was mixed too. Seven months after the compost amendment (December 2001), 1-year seedlings of downy oak were planted. At the stalk of each seedling, a 40 x 40 cm cellulose cover was laid on the ground to limit soil water loss and interspecific plant competition.

Compost

The six months maturity compost was produced by local company (Biotechna, Ensues, Southern Provence) and is certified in conformity with the [NF U 44-095 \(2002\)](#) norm for composts made with materials of sewage treatment origin. This compost was made with greenwastes (1/3 volume), pine bark (1/3 volume) and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 d at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in swathes. The swathes were mixed several times over the next 6 months to promote organic matter humification. The final compost was consistent with the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals (Table 1).



Photo 1. Experimental site at amendment time (May 2001).

Soil sampling and analysis

Soil samples were collected before compost amendment (May 2001, randomly in each future 200-m² plot), 7 months after amendment (December 2001), 1.5 years after amendment (December 2002) and 2.5 years after amendment (November 2003). After amendment, in each 200-m² plot, four soil samples corresponded to four plantation rows: one in the top, two in the middle and one at the bottom of the plot. Each soil sample was a mix of two samples collected at the stalk of 2 seedlings (within a 40 cm diameter circle, in the area covered by the cellulose cover). All the accessible soil down to bedrock was collected. The soil samples were oven dried at 40°C to constant weight and sieved through a 2 mm mesh before analysis. Soil pH_{H2O} was measured using 10 g of dried ground soil added to 25 ml of distilled water (NF ISO 10390, AFNOR, 1999; PHM 240 Radius meter). Three-fraction granulometry was determined gravimetrically (NF X 31-107, AFNOR, 1999). Cation exchange capacity (CEC) was measured by soil percolation with an ammonium acetate solution (NF X 31-130, AFNOR, 1999) and organic C was determined using sulphuric-chromic oxidation and spectrophotometry (NF ISO 14235, AFNOR, 1999; Cary 50 VARIAN). Soil organic matter concentration was calculated as 1.72 x organic carbon (C). Exchangeable potassium (K), calcium (Ca) and magnesium (Mg) were 1M NH₄Ac extracted and analysed by atomic absorption spectrophotometry (NF X 31-108, AFNOR, 1999; FS220 VARIAN). Total nitrogen (N) was determined by dry combustion and thermic conductimetry (ISO 13878, AFNOR, 1999; FP 428 LECO). Total phosphorus (P) and iron (Fe) were measured according to NF EN ISO 11885 (VARIAN VISTA Radial), and exchangeable P was determined in an sodium hydrogenocarbonate solution using spectrophotometry (Olsen *et al.*, 1954) (NF EN ISO 11263, AFNOR, 1999; Cary 50 VARIAN). Cu and Zn exchangeable forms were extracted using EDTA and concentrations were assessed by atomic absorption spectrophotometry (NF X 31-120, AFNOR, 1999; VARIAN VISTA Radial). Boron extractable in boiling water was determined by plasma emission spectrophotometry (X 31-122, AFNOR, 1993). To measure total concentrations of cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn), soil samples were digested in *aqua regia* and analysed using plasma emission spectrophotometry (NF EN ISO 11466, AFNOR, 1999). Mercury (Hg) was extracted according to NF X31-151 and determined using plasma emission spectrophotometry (VARIAN VISTA Axial).

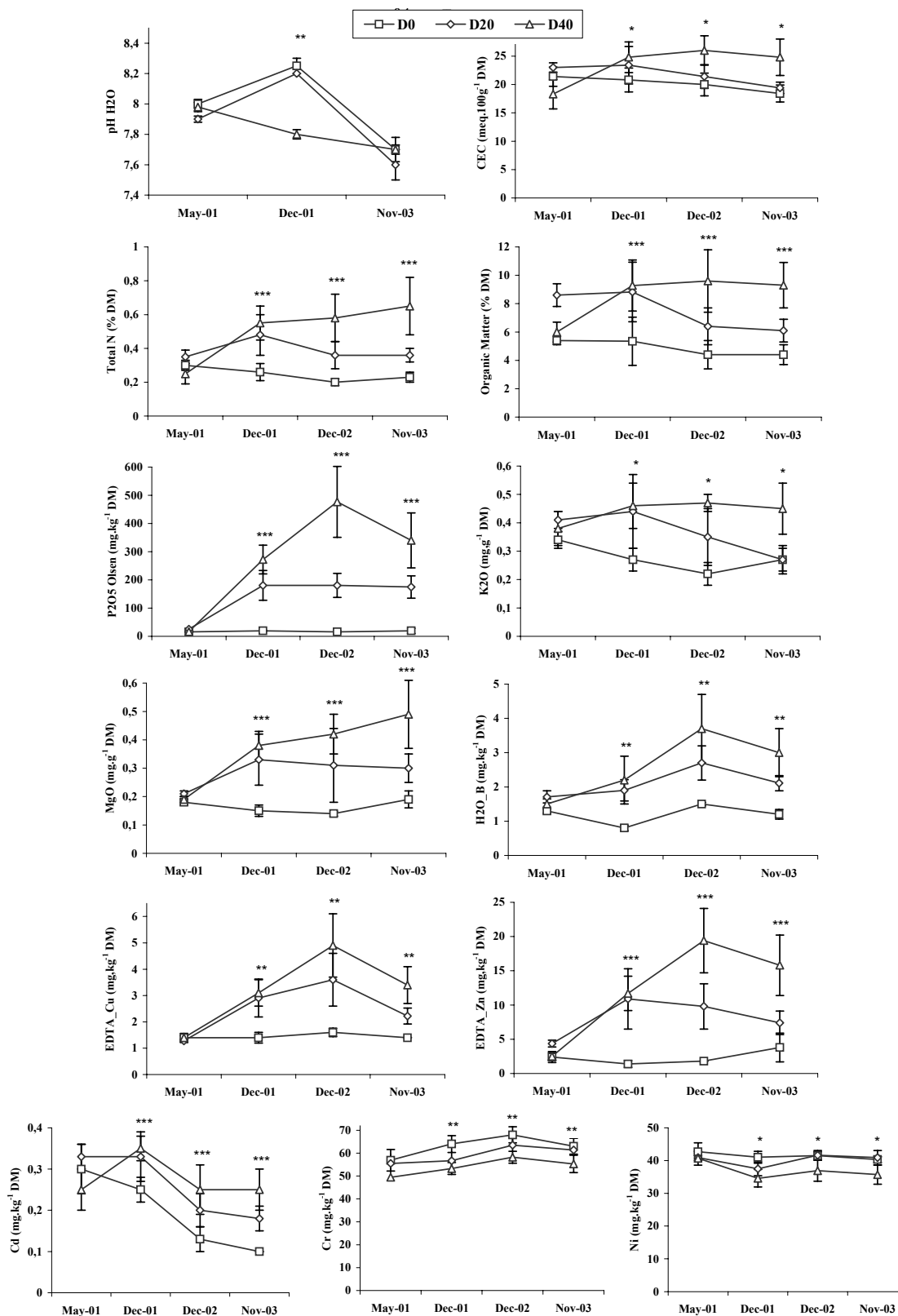


Figure 1. Soil properties before amendment (0), 7 months after amendment (at plantation time in December 2001), 18 months (December 2002) and 30 months (November 2003) after amendment for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40) plots. Mean \pm SE, N=4. *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$.

Seedling survival, growth and foliar chemical analysis

Seedling annual survival was determined one year after plantation (December 2002) and two years after plantation (November 2003) in the 10 plantation rows. Seedling height and basal diameter were measured in 4 rows per plot (2 seedlings per row), when planted (December 2001), 1 year after plantation (December 2002) and 2 year after plantation (November 2003). Leaves were collected in 4 rows per plot (2 seedlings per row) for N, P, cations and trace metal analysis, in mid October 2002 and early November 2003. The foliar material was washed with demineralized water, oven dried at 40°C and crushed (1 mm) in a trace metal free grinder (made with tungstene carbure, FOSS TECATOR Sample Mill 1093 Cyclotec). For N, K, Ca and Mg analysis, 250 mg of sample were digested one night in 5 ml H₂SO₄ (96%) and 5 ml H₂O₂ (35%), then heated at 400°C during 3 hours (Bioblock Scientific Digestion Unit 10401). The solutions were diluted 500 times by adding ultrapure water (Millipore Symplicity 185), filtered at 0.45µm and analysed by ion chromatography (Dionex DX120) (method adapted from [Masson and Andrieu, 1996](#)). The eluent used was 26 mmol.L⁻¹ methan sulfonic acid. Total concentrations of the other elements, P, B, Cd, Cr, Cu, Ni, Pb and Zn, were measured after digestion in *aqua regia* using atomic absorption spectrometry.

Statistical analysis

When the conditions of normality and variance homogeneity were checked (verified by Shapiro-Wilks and Bartlett tests respectively), data were interpreted using analysis of variance and means were compared using least significant different values (Tukey's test, 95 % level significance) ([Zar, 1984](#)). In case of non-homogeneity of variance, the Kruskal-Wallis test was used followed by the Newman-Student-Keuls test. Khi² test was used to interpret seedling survival rates (99 % level significance). The software Statgraphics plus (version 2.1: Statistical Graphics Corporation, © Copyright 1994-1996) was used.

Results

Compost effect on soil physical and chemical properties (Figure 1)

Compost significantly increased soil organic matter content and cation exchangeable capacity, while it significantly decreased soil pH. Compost effect on OM, CEC and pH was only

Table 2. Downy oak annual survival 1 year (December 2002) and 2 year (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40). Mean (SE), N=10 plantation rows. Results of the comparison are given by an exponent letter: values that do not differ at the 0.01 level are noted with the same letter.

| | seedling annual survival (%) | |
|-----|------------------------------|-----------------------|
| | 2002 | 2003 |
| D0 | 99 (1.6) ^a | 96 (2.4) ^a |
| D20 | 100 (2.2) ^a | 93 (3.2) ^a |
| D40 | 98 (1.5) ^a | 98 (2.3) ^a |

Table 3. Downy oak height and basal diameter 1 year (December 2002) and 2 year (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40). Mean (SE) , N=8. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter.

| | | height (cm) | basal diameter (cm) |
|----------|-----|-------------------------|------------------------|
| | | | |
| Dec 2001 | D0 | 29.4 (1.8) ^a | 4.4 (0.3) ^a |
| | D20 | 29.8 (2.3) ^a | 4.4 (0.4) ^a |
| | D40 | 28.7 (1.7) ^a | 4.7 (0.1) ^a |
| Dec 2002 | D0 | 30.4 (1.7) ^a | 5.2 (0.2) ^a |
| | D20 | 32.6 (2.3) ^a | 5.1 (0.4) ^a |
| | D40 | 28.4 (3.1) ^a | 5.8 (0.5) ^a |
| Nov 2003 | D0 | 30.6 (2.7) ^a | 6.1 (0.5) ^a |
| | D20 | 33.0 (2.6) ^a | 6.0 (0.5) ^a |
| | D40 | 30.6 (2.9) ^a | 5.9 (0.4) ^a |

Table 4. Nutrient concentrations in downy oak leaves 1 year (December 2002) and 2 year (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40). Mean (SE) , N=4. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter.

| | | N | P | K | Mg | Ca | B |
|------|-----|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | | % DM | % DM | % DM | % DM | % DM | mg.g ⁻¹ DM |
| 2002 | D0 | 1.53 (0.1) ^a | 0.11 (0.01) ^a | 0.43 (0.03) ^a | 0.16 (0.01) ^a | 1.35 (0.25) ^a | 22.2 (4.2) ^a |
| | D20 | 1.42 (0.1) ^a | 0.16 (0.02) ^b | 0.45 (0.08) ^a | 0.15 (0.03) ^a | 1.09 (0.15) ^a | 32.2 (6.9) ^{ab} |
| | D40 | 1.60 (0.1) ^a | 0.16 (0.01) ^b | 0.54 (0.08) ^a | 0.17 (0.01) ^a | 1.24 (0.20) ^a | 54.4 (8.2) ^b |
| 2003 | D0 | 1.21 (0.11) ^a | 0.10 (0.01) ^a | 0.27 (0.02) ^a | 0.13 (0.01) ^a | 1.07 (0.1) ^a | 8.4 (2.8) ^a |
| | D20 | 1.48 (0.13) ^a | 0.16 (0.02) ^b | 0.34 (0.04) ^a | 0.16 (0.01) ^a | 1.12 (0.13) ^a | 11.3 (2.7) ^a |
| | D40 | 1.24 (0.04) ^a | 0.16 (0.01) ^b | 0.29 (0.01) ^a | 0.15 (0.01) ^a | 1.12 (0.13) ^a | 13.1 (0.4) ^a |

Table 5. Trace metals concentrations in downy oak leaves 1 year (December 2002) and 2 year (November 2003) after plantation for control (D0), 20 kg of compost per seedling (D20) and 40 kg of compost per seedling (D40). Mean (SE), N=4. Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter.

| | | Cd | Cr | Cu | Ni | Zn |
|------|-----|----------------------------|------------------------|-------------------------|--------------------------|--------------------------|
| | | mg.kg ⁻¹ DM | mg.kg ⁻¹ DM | mg.kg ⁻¹ DM | mg.kg ⁻¹ DM | mg.kg ⁻¹ DM |
| 2002 | D0 | 0.045 (0.009) ^a | <0.3 | 1.6 (0.6) ^a | <0.1 | 166 (18.1) ^{ab} |
| | D20 | 0.05 (0.03) ^a | <0.3 | 2.9 (0.9) ^a | <0.1 | 221 (43.7) ^b |
| | D40 | 0.05 (0.01) ^a | <0.3 | 3.2 (0.7) ^a | <0.1 | 113 (5.4) ^a |
| 2003 | D0 | <0.01 | 1.7 (0.6) ^a | 8.9 (1) ^a | 0.88 (0.15) ^a | 249 (36.1) ^a |
| | D20 | <0.01 | 1.4 (0.2) ^a | 11.8 (2.4) ^a | 0.63 (0.06) ^a | 299 (41.5) ^a |
| | D40 | <0.01 | 2 (0.8) ^a | 7.6 (0.5) ^a | 1.1 (0.5) ^a | 287 (96.3) ^a |

significant on D40 amended plots. Two and a half years after amendment, the compost's reductive effect on D40 soil pH had disappeared. Soil OM content and CEC followed a similar pattern. They increased 7 months after amendment on D40 and D20 plots, and remained stable on D40 plots until 2.5 years after amendment, while they decreased on D20 plots.

Compost significantly increased nutrient and oligoelement concentrations in soil. Total N, exchangeable P, K, Mg, B, Cu and Zn concentrations were higher on amended plots (D20 and D40) compared to control (except for K for which only the D40 effect was significant). Thus, compost at maximum rate overall increased soil nutrient and oligoelement concentrations by a factor of 2 to 3, except for exchangeable P and Zn. Exchangeable P concentrations in D40 and D20 plots were respectively 20 and 10 fold higher than on control plots, 10 and 5 fold higher for exchangeable Zn. Both compost rates induced these increases, and D40 soil concentrations were higher than D20.

The compost's enhancement effect on total N, exchangeable Mg and K concentrations remained 2.5 year after amendment for D40. In contrast, exchangeable Cu, Zn, B and P reached maximum concentrations values 1.5 years after amendment on D40 plots and then decreased 2.5 years after amendment. The same pattern was found on D20 plots, except for exchangeable Zn, which reached its maximum concentration 7 mo after amendment on D20 plots.

Compost effect on soil heavy metal contamination

Compost significantly increased soil total Cd concentrations at both rates, while it significantly decreased total Cr and Ni. Two and a half years after amendment, Cd total concentration in D40 and D20 amended soils remained higher than control, D40 being the highest (x 1.5 control). Compost dilution effect on soil total Cr and Ni concentrations disappeared 2.5 year after amendment on D20 for Ni, but remained on D40 plots for both elements.

Compost effect on downy oak seedlings

Compost had no significant effect on seedling survival, either in 2002, or in 2003. Survival rates were higher the first year after planting (2002) than the second (2003) (Table 2).

No effect of compost was noted on seedling height and seedling basal diameter in 2002 and 2003 (Table 3).

Compost had no significant effect on N and cation concentrations in downy oak leaves in 2002 (Table 4), even if N and K concentrations in D40 plot were higher compared to control. In 2003, no significant differences were reported for N and cations between treatments. However, P foliar concentration in D40 plots was significantly higher than control both in 2002 and 2003. Likewise, in 2002, B concentration in seedling leaves were significantly higher (x 2.5) in D40 amended plot compared to control, while this effect disappeared in 2003 (Table 4). That year, macronutrient and B concentrations in seedlings were lower than in 2002.

Although compost amendment increased total Cd, exchangeable Cu and Zn concentrations in soil, it did not induce significant higher concentrations of these trace metals in downy oak seedling leaves, except for Zn on D20 plot in 2002. Moreover, it had no effect on Cr and Ni concentrations in seedlings (Table 5).

Discussion

Compost effect on soil properties and trace metal contamination

Compost significantly increased soil organic matter content and cationic exchange capacity. Similarly, Guerrero *et al.* (2001) found that soil total organic carbon increased after municipal waste compost amendment, although their application rates were lower (1 kg, 2 kg.m⁻²) than ours (20 kg, 40 kg.m⁻²). As OM provides humic substances rich in –COOH or –OH fixation sites for cations (Baize, 1988), the compost led to a CEC increase. Moreover, the compost effect on these 2 parameters remained stable until the end of the experiment for D40 rate, which clearly indicates that the maximum rate provided a durable improvement of soil fertility. OM is a source of plant nutrients and its progressive mineralization provides bioavailable ions to plants.

Compost significantly decreased soil pH 1.5 years after amendment. Compost pH was slightly lower than the calcareous soil pH. Yet soil pH is a major factor influencing the absorption of nutrients by plants (Khanna and Ulrich, 1984). Kabata-Pendias and Pendias (1992) reported that the most mobile fractions of ions in soils occur at lower range of pH. Thus, this compost reductive effect on pH may improve seedling nutrition by increasing nutrient availability. However, 2.5 years after amendment, the compost effect on pH has disappeared.

Compost overall increased nutrient and oligoelement concentrations and availability in amended soils. Likewise, several authors reported an increase in total and exchangeable salts in amended soils (Guerrero *et al.*, 2001; Brockway, 1983; Martinez *et al.*, 2003a). Total N, exchangeable P, K, Mg, B, Cu and Zn concentrations increased with the compost rate. Moreover, unlike Martinez *et al.* (2003a), compost enhancement effects remained significant at the end of the experiment for N, P, Mg, B, Cu (both rates), and K, Zn (D40), even if a decrease with time for some elements was noted. Thus the applied rates are sufficient to provide seedling fertilization until the end of the 2nd year of plantation.

Exchangeable P and Zn concentrations respectively increased by a factor 20 and 10 in D40 plot compared to control, whereas the other elements brought by compost only increased by a factor 2 to 3. Bingham (1966) reported that exchangeable P concentrations of 40 ppm (Olsen method) in agricultural soils were relatively high. In our experiment, maximal exchangeable P concentration in D40 amended soil was higher than 450 ppm. Likewise, Korboulewsky *et al.* (2002b) found that P significantly increased in the top soil and subsoil of a vineyard after compost amendment and concluded that this element was the limiting factor for sewage sludge compost use. The sewage sludge of the compost comes from a sewage plant that applies a dephosphatation process to sewerage. Thus P is concentrated in the resulting sludge, and so in compost. Moreover, Martinez *et al.* (2003b) showed that the PO₄-P concentration in run-off water increases in biosolid treated plots. Thus, as this element can be easily leached to surface or groundwater, and as the vegetation absorption is negligible compared to the remaining concentration in the soil, its excessive increase could lead to serious water pollution and eutrophication and limits the rates of compost which can be used.

Likewise, Zn is considered to be readily soluble relative to the other heavy metals in soils (Kabata-Pendias and Pendias, 1992) and its exchangeable form increasing in soil after compost amendment could induce some water pollution. Moreover, this element is reported to be toxic at lower levels than the other metals for soil microorganisms (10 mg.kg⁻¹) (Kabata-Pendias and Pendias, 1992) and its increase in soil could consequently decrease soil fertility by depleting decomposition processes. However, this effect disappeared for Zn 2.5 years after amendment, whereas it remained for P, suggesting that it is the most limiting factor for compost use.

Compost increased soil total Cd concentration in amended plots and this effect remained at the end of the experiment. This result differs from that of Martinez *et al.* (2003a) who found no significant Cd increase in soil after biowaste application. However, Cd concentration

increase in D40 soil was slight (x 1.5 compared to control), and the 0.35 mg.kg⁻¹ concentration reached 7 mo after amendment remained lower than reported mean soil Cd concentrations in the literature (Kabata-Pendias and Pendias, 1992).

Moreover, total Cd concentration in soil decreased over time in all the treatments including control. This decrease might be due to the site preparation at the time of compost amendment: removal of vegetation on seedling plantation holes and soil mixing. This may have induced soil destructure and increase its erodibility, enhancing deep or surface leaching of Cd in dissolved, particulate- or organic ligands-adsorbed forms (Kabata-Pendias and Pendias, 1992).

Compost decreased Ni and Cr total concentrations in amended soils compared to control. Before amendment, the soil had relatively high Cr and Ni concentrations (Table 1) if compared to the data of De Nicola *et al.* (2003). This is in accordance with the results of Hernandez *et al.* (2003) on French soil contamination by trace elements, as the studied area is exposed to significant acid atmospheric pollution (AIRMARAIX, 1999). And the compost used was less concentrated than the soil for these 2 elements. Thus the mix of compost and soil induced a decrease in Ni and Cr total concentrations, which will remain until the OM of compost is mineralized.

Compost effect on Quercus pubescens seedlings

Despite its overall enhancing effect on soil fertility, compost had no effect either on *Quercus pubescens* survival, or on its growth. These results were not due to poor adaptation of *Quercus pubescens* to the site conditions. This species naturally occurs on slope, below the experimental plots. Moreover, more than 93 % of seedlings were still alive after the very dry summer of 2003, which indicates that the trees were well adapted to the environmental conditions. These results differ from those reported from other fertilization experiments on other tree species in the literature. Several authors (McKay and Moffat, 2001; Brockway, 1983; Dutch and Wolstenholme, 1994) reported that sewage sludge amendment increased growth of *Picea sitchensis* or *Pinus densiflora*. On the one hand, *Quercus pubescens* may poorly respond to compost fertilization. However, Berger and Glatzel (2001) showed that organic N fertilizer led to maximum biomass of *Quercus petraea* seedlings only by means of maximum coarse and fine root masses. Thus, *Quercus pubescens* could have responded to compost amendment by increasing root biomass, but we did not study this parameter. On the other hand, the cellulose cover laid at seedling stalks for the 3 treatments might have greatly

improved the seedlings' living conditions by increasing soil moisture. Therefore, *Quercus pubescens*, which is sensitive to soil water content (Damesin *et al.*, 1998) may have uniformly responded to this experimental set-up, explaining the high survival rates, whereas aerial growth was not affected by compost amendment.

Compost seemed to slightly increase seedling foliar N and K concentrations the first year after plantation whereas this effect did not remain in 2003. Rapp *et al.* (1999) showed that an increase in available nutrients accelerated their uptake by 2 deciduous oaks (*i.e.* *Quercus pyrenaica* and *Quercus lanuginosa*) and their accumulation in leaves. Similarly, Berger and Glatzel (2001) reported that N fertilization increased N foliar content of *Quercus petraea* seedlings. But these authors also found that N fine root content was a better indicator of N inputs than foliar content. This could explain the lack of significance of N and K foliar increase in 2002. Moreover, as the *Quercus pubescens* leaves were collected at the beginning of autumn, the seedlings might have begun to retranslocate nutrients from leaves to perennial organs (Damesin *et al.*, 1998) before senescence, especially N. This could have decreased N and K foliar concentrations and consequently our results are not significant for these 2 elements.

However, the compost enhancement effect on soil exchangeable P induced a significantly higher foliar P concentration in seedlings grown on D40 amended plot for both first and second years after plantation. If P had been a limiting element in the experimental soil for *Quercus pubescens*, its increase in this species' leaves should have been followed by increased growth in D40 plot. As no effect of compost on seedling aerial growth was found, this element might not be limiting in the experimental soil for this species. The control soil might provide sufficient P to seedlings, and luxury consumption of this element might be observed for seedlings grown on D40 plot (Chapin, 1980).

As for P, compost induced higher foliar B concentration in D40 amended plot in 2002. Kabata-Pendias and Pendias (1992) reported that P and B have similar reactions with -OH groups in soil and the uptake of these elements by plants is likely to follow similar patterns. As B is an essential plant nutrient (Bradford, 1966), this should have improved the growth of *Quercus pubescens*. But it seems that this element, as for P, was not limiting in the soil before amendment for this species.

As for N and K, this enhancing B effect disappeared in 2003. Moreover, in 2003, macronutrients and B concentrations in seedling leaves were overall lower than in 2002. Over a period of water stress, such as summer 2003, a strong decrease in nutrient uptake can occur.

This is due to a decreased transfer of ions to the root associated with reduced plant assimilation caused by water stress (Bowen, 1984).

Compost had no significant effect on foliar Mg concentration although compost increased this element's concentration in amended soils. This might have been due to the formation of humates and fulvates between Mg and the organic matter of the compost. So this element could be retained in a form which makes it unavailable to plants (Moreno *et al.*, 1996).

Seedling contamination by compost trace metals

Compost had no significant effect on seedling foliar contamination by trace metals, despite the increased Cd and Cu concentrations in the amended soil. In calcareous soil, the high pH favours the immobilization of most heavy metals (Martinez *et al.*, 2003a) and even if compost induced a decrease in soil pH, it remained very basic and may have protected seedlings from Cd and Cu absorption. Likewise, Planquart *et al.* (1999) showed that nutrient and trace metal content in plants are more influenced by soil nature than compost application. However, foliar Cu concentration increased by a factor of 2 in D40 amended plot compared to control in 2002, even if this is not significant. Furthermore, Cu accumulates preferentially in plant root rather than in shoot (Wisniewski and Dickinson, 2003; Van Den Driessche, 1984) and consequently leaves may not be a good indicator of seedling contamination. Thus, this element could have accumulated in seedling root.

The only trace element that significantly increased in *Quercus pubescens* leaves was Zn in 2002. However, the 2 rates induced opposite effects on Zn foliar concentration: maximum Zn foliar concentration was obtained in D20 plot, whereas it was the lowest in D40 plot. Kabata-Pendias and Pendias (1992) reported that Zn availability is negatively correlated with P compounds present in soils and the immobilization of this element in soils rich in Ca and P has an important practical impact on the Zn deficiency of plants. Likewise, Zwonitzer *et al.* (2002) showed P to influence Zn bioavailability to plants. Thus, in D40 plot, the dramatic increase of P concentration may have limited Zn absorption by seedlings, despite its increase in soil. However, P concentration in D20 plot might not have reached a sufficient concentration to decrease Zn absorption. Moreover, Kabata-Pendias and Pendias (1992) also reported that interactions between P and B occur in soils and these interactions are linked with Zn absorption by plants. Thus, B, which significantly increased in D40 soil and seedling leaves in 2002, might have played a role in the decreased absorption of Zn by seedlings in D40 amended plot.

Conclusion

Compost did not improve *Quercus pubescens* growth on the experimental site, despite a higher soil fertility on amended plots. Seedlings were well adapted to site conditions as survival for both experimentation years was greater than 93 %. The experimental set-up (*i.e.* cellulose cover at seedling stalk) may have greatly improved the seedlings' living conditions (by limiting soil water loss), as *Quercus pubescens* is very sensitive to soil water content.

Foliar P and B increased in amended seedlings, whereas growth was similar for all treatments, suggesting luxury consumption for these 2 elements. Moreover, despite an increase in soil total Cd, exchangeable P and Zn concentrations after amendment, seedling leaves were not significantly contaminated by these trace elements. However, exchangeable P and Zn concentrations strongly increased in D40 amended soil, respectively by a factor 20 and 10, and could induce water pollution and eutrophication. Therefore, compost use for reforestation may be hazardous, especially in sloping areas, and should be limited to fertilization-sensitive species.

Acknowledgements

This research was commissioned by the Conseil Général des Bouches-du-Rhône (France), and supported by the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie), the Provence-Alpes-Côte-d'Azur Region and the French Rhône-Méditerranée-Corse Water Agency.

Résultats

L'apport de compost améliore durablement la fertilité du sol pour les deux doses choisies (20 et 40 kg de compost brut par plant). La teneur en matière organique, la capacité d'échange cationique et les concentrations en macronutriments (N, P, K, Mg) et oligoéléments (B, Cu, Zn) augmentent après épandage proportionnellement à la dose de compost. De plus, le pH diminue légèrement un an et demi après épandage.

Cependant, les teneurs en P et Zn sont respectivement multipliées par 20 et 10 sur D40 par rapport au témoin. Comme ces deux éléments sont assez facilement mis en solution et que leur absorption par la végétation est négligeable par rapport à leur teneur édaphique, leur augmentation excessive dans le sol pourrait entraîner des phénomènes de pollution des eaux et d'eutrophisation.

Par ailleurs, les résultats obtenus concernant les métaux traces diffèrent en fonction de l'élément considéré. Ainsi, le mélange du compost au sol augmente légèrement la concentration en Cd du sol à D40, alors qu'il diminue celle du Ni et du Cr par dilution. Cependant, la concentration en Cd atteinte sur D40 reste en dessous des teneurs moyennes généralement trouvées dans les sols.

Malgré les effets fertilisants globaux du compost sur le sol, l'amendement n'améliore pas la survie de *Q. pubescens*, et ne stimule pas sa croissance. L'inadéquation des conditions environnementales du site peut être écartée, puisque des individus poussent naturellement dans la pente où se trouvent les parcelles. D'autre part, quelque soit le traitement, plus de 93 % des plants sont vivants deux ans après plantation, et ont survécu à la sécheresse de l'été 2003. L'hypothèse la plus vraisemblable est que la protection cellulosique (paillage) disposée au pied de chaque plant a joué un rôle prépondérant dans l'amélioration des conditions de vie de *Q. pubescens*, probablement en augmentant le taux d'humidité du sol. Cette espèce semble plus sensible à ce facteur humidité du sol qu'à sa teneur en éléments fertilisants.

Le compost a induit une augmentation des teneurs foliaires en P, B et Zn. Par ailleurs, aucune contamination des plants en Cd et Cu n'a été mise en évidence, malgré l'augmentation de ces éléments dans le sol.

SYNTHESE DE LA DEUXIEME PARTIE

Dans le cas d'un site dégradé par le passage répété du feu, après un suivi de deux années, l'utilisation de compost pour le reboisement est plutôt favorable en plantation forestière. En effet, l'apport de compost améliore la fertilité globale du sol et stimule l'absorption des nutriments et la croissance des plants. De plus, le meilleur statut nutritionnel des plants poussés sur compost pourrait être responsable de la meilleure résistance des jeunes arbres à la sécheresse observée en 2003 sur parcelles amendées.

Cependant, les réponses des 4 espèces plantées ne sont pas homogènes et se distinguent les unes des autres en fonction de leurs exigences écologiques, et de la dose de compost. Les chênes, à croissance lente et longue durée de vie (stratégie k), sont beaucoup moins sensibles à l'apport de compost que les pins, à croissance rapide et durée de vie plus courte (stratégie r) vis-à-vis de leur croissance. L'effet stimulant du compost sur la croissance est inexistant pour *Q. pubescens*, faible pour *Q. ilex*, moyen pour *P. pinea* et maximal pour *P. halepensis*.

Par ailleurs, l'effet favorable du compost sur la survie des plants en période de sécheresse apparaît pour *Q. ilex* et *P. pinea*, alors que *P. halepensis* et *Q. pubescens* y sont insensibles. L'humidité du sol semble être le facteur prépondérant contrôlant la survie de *Q. pubescens*, et le paillage cellulosique disposé au pied des plants a probablement été très bénéfique pour cette espèce. *P. halepensis* est une espèce pionnière des conditions écologiques drastiques, tolérante au stress (Michalet 2001, in Quézel & Médail 2003) et adaptée aux milieux xériques. La sécheresse de 2003 a probablement peu affecté sa survie.

Enfin, la stimulation de la croissance après amendement n'est pas proportionnelle à la dose de compost, sauf pour *P. pinea*. Il semble que les fortes teneurs en phosphore du sol sur D40 (autour de 400 mg.kg⁻¹) ont atteint un seuil toxique pour *P. halepensis* et *Q. ilex*. Ainsi, cet élément apparaît limitant pour l'utilisation de compost à la dose D40, même si l'effet sur la croissance des plants et l'amélioration des taux de survie de *Q. ilex* en 2003 ne sont notés que pour cette dose.

L'apport de compost à la dose D40 semble également mal adapté car les fortes quantités de P enregistrées dans le sol après amendement peuvent localement poser des problèmes de contamination des eaux de surface et des eaux souterraines. Ce phénomène est lié à l'utilisation de boues issues d'un traitement tertiaire de déphosphatation des eaux usées, boues très riches en P, pour la fabrication du compost. Ainsi le phosphore apparaît à nouveau

comme l'élément limitant pour l'utilisation de ce type de compost en milieu naturel. Mais, dans le cas de la plantation sur le plateau de l'Arbois, l'apport de compost localement au pied de chaque arbre, couplé avec une faible densité de plantation (400 plants par hectare), permet de limiter grandement ce risque. Par ailleurs, la concentration en P échangeable atteinte dans le sol amendé à D40 (450 ppm) est plus de 10 fois supérieure à la concentration reportée comme forte dans les sols agricoles par [Bingham \(1966\)](#) (40 ppm). L'épuisement de cette ressource énorme de phosphore se fera certainement très progressivement et restreint fortement le renouvellement d'épandages sur la zone expérimentale.

Le deuxième élément qui limite la quantité de compost à épandre est le Zn, et dans une moindre mesure le Cu, du fait de la richesse du compost en ces éléments. De la même manière que le P, le Zn est un élément facilement mis en solution qui peut poser des problèmes de contamination des eaux, notamment à la dose D40. Cependant, lorsqu'on considère la dose réelle d'apport de compost brut à l'hectare ($16 \text{ t}\cdot\text{ha}^{-1}$) correspondant à D40, les risques de pollution des eaux par le zinc deviennent faibles. Par contre, cet élément est toxique pour les microorganismes du sol à des doses faibles ($10 \text{ mg}\cdot\text{kg}^{-1}$) comparativement aux autres éléments traces métalliques, et l'élévation importante de sa teneur édaphique après amendement pourrait perturber les processus de minéralisation de ce compartiment.

Malgré le fait que le compost apporte un supplément de Ni, Cr, Pb et Cd au sol, aucune contamination significative n'a été mise en évidence pendant la durée de l'expérimentation, du fait de la concentration faible du compost utilisé en ces éléments. Au contraire, l'amendement induit une dilution provisoire du sol en chrome et nickel. Ce phénomène peut favoriser la bonne installation des plants. Mais cet effet disparaît dès la deuxième année après l'épandage, suite à la minéralisation progressive de la matière organique apportée par le compost. D'autres changements des conditions physico-chimiques du sol pourront induire la remobilisation de ces éléments persistants dans le sol et augmenter leur disponibilité pour les plants, conduisant à des phénomènes d'accumulation et de toxicité.

Il semble que la nature calcaire du sol expérimental, qui « piège » les cations métalliques, a un effet protecteur sur les plants en diminuant l'absorption des éléments traces métalliques, même si leur teneurs augmentent après épandage dans le sol.

Les effets du compost semblent plus durables dans la pente que sur le plateau, probablement du fait d'une minéralisation plus rapide de la matière organique du compost sur ce dernier. D'autre part, les fortes teneurs en P et Zn du sol amendé à D40 orientent l'utilisation de compost vers des zones de plateau où le ruissellement est moindre, et implique une faible densité de plantation pour limiter la possible contamination des nappes et des cours d'eau, surtout en région calcaire karstique.

SYNTHESE GENERALE

Synthèse

Cette étude apporte une vision globale du fonctionnement du système garrigue, qui met en avant une stabilité rapidement acquise après incendie (7 ans) vis-à-vis des cycles des éléments majeurs (N et P). L'apport de compost, facteur exogène multi-effets (fertilisation, écrasement, dépôt d'une couche fertile) exacerbe les réponses des végétaux et révèle leurs différentes stratégies de gestion des nutriments et de productivité. Les traits de vie des espèces, ainsi que les stratégies régénératives conditionnent la réactivité des plantes, du point de vue intensité et rapidité de réponse. Ces considérations permettent d'approcher l'importance relative des espèces sur le fonctionnement de l'écosystème, d'identifier les espèces à rôle clef sur les cycles des éléments, et de visualiser la complémentarité d'utilisation des ressources qui existent au sein d'un système naturel.

Cependant, malgré la réactivité constatée des espèces de garrigue à l'augmentation du niveau de ressources après amendement, on peut se demander si ce système à faible productivité (comparativement à un jeune système forestier par exemple) est approprié pour le recyclage des éléments apportés par le compost aux doses choisies.

D'une manière générale, l'apport de compost de boues et de déchets verts en milieu naturel de garrigue est plutôt favorable à la reforestation. D'une part, il a un effet direct mélioratif sur la croissance et la nutrition des espèces arborées, mais surtout sur leur survie en période de sécheresse intense (plantation). D'autre part, l'apport en surface permet la génération d'îlots au sein de la garrigue, où une succession végétale plus rapide peut s'initier. Le terme de « microsuccession » semble adapté à cette situation locale. Au sein de ces îlots, la diversité fonctionnelle et les processus de la dynamique de végétation sont stimulés par des espèces qui dynamisent les cycles des éléments et pourraient rendre l'environnement plus favorable à l'implantation d'espèces des stades successifs suivants (modèle de facilitation, Connell et Slatyer, [Quézel & Médail 2003](#)). En outre, [Lepart & Escarré \(1983\)](#) notent que le blocage de *Q. coccifera* sur la dynamique de la végétation après le passage du feu est levé au moment de la sénescence des individus, qui produit l'ouverture des formations et permet l'installation des ligneux hauts. Dans quelle mesure la couche de compost déposée sur le sol entre les zones recouvertes par *Q. coccifera* peut-elle recréer ces conditions d'ouverture du milieu favorables à la colonisation par les ligneux ? Au vu des effets positifs du compost sur *P. halepensis*, cette espèce colonisatrice pourrait s'installer à plus ou moins long terme au sein de ces îlots et initier le développement de la strate arborée.

D'autre part, en garrigue, le compost tend à rétablir l'équilibre entre semenciers et espèces à rejet de souche dominantes, en favorisant le développement des premières, ce qui va dans le sens d'une augmentation de la résilience globale du système après incendie.

Cependant, l'épandage de compost devrait être limité aux deux doses intermédiaires utilisées dans la plantation (8 t.ha⁻¹) et la garrigue (50 t.ha⁻¹). Celles-ci sont suffisantes pour induire un effet bénéfique sur la plupart des paramètres suivis. L'utilisation du compost aux doses maximales (16 et 100 t.ha⁻¹) est à exclure du fait des trop grandes quantités de phosphore qu'elles introduisent dans le milieu naturel. D'une part, cet élément peut créer des problèmes de contamination des eaux, même si durant les deux ans d'expérimentation il semble fixé en grande partie par le sol calcaire. D'autre part, il se trouve en excès par rapport aux autres nutriments et peut engendrer des phénomènes de toxicité pour certaines espèces de plantes sensibles. Par ailleurs, l'apport de compost à dose intermédiaire limite l'enrichissement du milieu naturel en éléments trace métalliques, polluants persistants, surtout en zinc, deuxième élément limitant l'épandage de compost de boues après le phosphore. Les quantités importantes de ces deux éléments dans le compost ne permettent pas d'envisager des épandages successifs aux doses étudiées.

La nature calcaire du sol expérimental et la minéralisation lente du compost relativement mûre permettent de limiter l'exportation d'éléments vers les eaux et de limiter la contamination des plantes en éléments potentiellement toxiques.

Enfin, le mode d'épandage apparaît déterminant vis-à-vis des effets de la sécheresse sur les plantes. En effet, le mélange du compost au sol favorise la tolérance à ce facteur prépondérant en région méditerranéenne, alors que l'apport en surface augmente plutôt la vulnérabilité des plantes à la sécheresse.

Perspectives

Vis-à-vis de l'objectif de reboisement du milieu garrigue, il serait intéressant de compléter cette étude sur divers points :

- Tester les effets potentiellement favorables du compost sur la germination des espèces arborées, notamment du pin d'Alep.
- Suivre la durabilité des effets du compost sur les arbres de la plantation et mettre en place un suivi de la régénération naturelle possible (semenciers à proximité) du pin d'Alep dans la zone amendée de garrigue.
- Voir si l'apport de compost en surface protège également les plants arborés des effets négatifs de la sécheresse.

Pour accentuer la pertinence et la rigueur de l'étude, il serait opportun d'étudier séparément les effets des facteurs fertilisation et écrasement liés à l'épandage. Le premier facteur peut être isolé en milieu contrôlé, avec apport de compost sur des espèces cultivées en pot. Le deuxième nécessite la simulation *in situ* d'un épandage mécanique.

D'autres compartiments impliqués dans le fonctionnement de l'écosystème peuvent réagir à l'apport de compost, tels la microfaune, la mésofaune et la faune sauvage. Ces compartiments pourraient être pris en compte dans une étude élargie à l'écosystème dans son ensemble, en se focalisant notamment sur les transferts d'éléments vers certaines espèces clefs et sur leurs réactions écotoxicologiques.

L'exemple du phosphore souligne qu'il serait opportun de mettre en place des dispositifs de surveillance des teneurs en éléments dans les eaux de la nappe, pour les zones de plateau fracturées sur lesquelles s'installent généralement le *Q. coccifera*.

Dans l'éventualité où d'autres épandages en milieu naturel auraient lieu sur d'autres sites, il serait nécessaire de compléter la surveillance des possibles contaminations par la prise en compte des micropolluants organiques et des virus, en sus de celle des éléments trace métalliques.

D'autre part, ce type d'étude pourrait être élargi à des types d'amendements différents et des sols plus pauvres en nutriments, par exemple plus acides.

Des épandages à dose modérée pourraient être envisagés dans des contextes différents, dans un objectif de restauration. L'amélioration des potentialités de résilience après feu pourrait être testée juste après incendie, alors que la matière organique naturelle a été consommée en grande partie.

Par ailleurs, au vu des effets favorables du compost sur *B. retusum*, il serait intéressant de tester les potentialités d'un apport de compost à accélérer la création de pâturages coupe-feu dans des zones sensibles au feu colonisées par la garrigue.

Enfin, les aspects de sciences humaines associés à ce type de pratique viendraient compléter de manière pertinente cette étude. L'intérêt économique associé au recyclage des produits résiduels pourrait être chiffré et une évaluation des surfaces disponibles pour des épandages dans un but de reforestation sur des terrains fréquemment incendiés serait intéressante.

Par ailleurs, il est indispensable d'étudier l'acceptabilité sociale de telles pratiques d'épandage de produits résiduels en milieu naturel.

BIBLIOGRAPHIE

A

- ADEME** (1995). *Les micropolluants métalliques dans les boues résiduelles des stations d'épuration urbaines*. ADEME éditions, Paris. 103 pp.
- ADEME** (1999). *Evaluation des risques écotoxicologiques liés à la valorisation agricole des déchets et produits dérivés : synthèse des résultats obtenus sur plantes et microorganismes*. Deuxième rapport d'avancement, 84 pp.
- ADEME** (2000). *Composts de boues de stations d'épuration municipales : qualité, performances agronomiques et utilisations*. ADEME, 423 pp.
- Aerts R** (1995). *The advantages of being evergreen*. *Trends in Ecology & Evolution* 10(10), 402-407.
- Aerts R** (1997). *Nitrogen partitioning between resorption and decomposition pathways : a trade-off between nitrogen use efficiency and litter decomposibility ?* *Oikos* 80, 603-606.
- Aerts R & Van Der Peijl MJ** (1993). *A simple model to explain the dominance of low-productive perennials in nutrient-poor habitats*. *Oikos* 66(4), 144-147.
- AFNOR** (1999). *Qualité des sols*. AFNOR, Recueil de normes, Paris, Vol.1 et 2.
- Agassi M, Kirsten WFA, Loock AH & Fine P** (1998). *Percolation and leachate composition in a disturbed soil layer mulched with sewage biosolids*. *Soil Tillage Research* 45, 359-372.
- AIRMARAIX** (1999). *Campagne de mesures temporaire de la camionnette laboratoire : Aix les Milles, 20.05.1999- 8.07.1999*.
- Albiach R, Canet R, Pomares F & Ingelmo F** (2000). *Microbial biomass concentration and enzymatic activities after the application of organic amendments to a horticultural soil*. *Bioresource Technology* 75, 43-48.
- Archibold OW** (1995). *Mediterranean ecosystems*. *Ecology of world vegetation*, Chapman et Hall, London, 131-164.
- Arianoutsou M & Thanos CA** (1994). *Legumes in the fire-prone Mediterranean environment*. *Proceedings of the 2nd International Conference on Forest Fire Research*, Vol.II, D.19, Coimbra, 995-1005.
- Aubert G** (1983). *Apport de la connaissance de la végétation spontanée dans la recherche des potentialités forestières d'un territoire en région méditerranéenne*. *Revue Forestière Française*, XXXV(6), 425-442.

B

- Baeza MJ, Raventos J, Escarré A & Vallejo VR** (2003). *The effect of shrub clearing on the control of the fire-prone species Ulex parviflorus*. *Forest Ecology and Management* 186, 47-59.
- Baize D** (1988). *Guide des analyses courantes en pédologie*. Institut National de la Recherche Agronomique, Paris.
- Bagnouls F, Gaussen H** (1957). *Les climats biologiques et leur classification*. *Annales de Géographie* 355, 193-220.
- Baldy V, Gessner MO & Chauvet E** (1995). *Bacteria, fungi and the breakdown of leaf litter in a large river*. *Oikos* 74 (1), 93-102.
- Ballard R** (1984). *Fertilization of plantations*. *Nutrition of plantation forests* (eds GD Bowen & EKS Nambiar), Academic Press, London, 327-360.
- Ballini C** (1993). *Contribution à l'étude écologique des groupements à Ulex parviflorus Pourr. en Provence calcaire : régénération, structure, productivité et dynamique des bioéléments*. Thèse de doctorat, Université de Provence, Marseille.

- Ballini C & Bonin G** (1995). *Nutrient cycling in some Ulex parviflorus Pourr. scrubs in Provence (southeastern France). II. Nutrient release from decomposing litter*. European Journal of Soil Biology 31(3), 143-151.
- Barajas-Aceves M, Hassan M, Tinoco R & Vazquez-Duhalt R** (2002). *Effect of pollutants on the ergosterol concentration as indicator of fungal biomass*. Journal Microbiol. Methods 50, 227-236.
- Barbero M** (1990). *Méditerranée: bioclimatologie, sclérophyllie, sylvigénèse*. Ecologia Mediterranea XVI, 1-12.
- Barbero M, Bonin G, Loisel R, Miglioretti F & Quézel P** (1987). *Impact of forest fires on structure and architecture of mediterranean ecosystems*. Ecologia mediterranea XIII, 39-50.
- Barbero M, Bonin G, Loisel R & Quézel P** (1990). *Changes and disturbances of forest ecosystems caused by human activities in the western part of the mediterranean basin*. Vegetatio 87, 151-173.
- Basta NT & Tabatabai MA** (1985). *Determination of total potassium, sodium, calcium, and magnesium in plant materials by ion chromatography*. Soil Sci. Soc. Am. J. 49, 76-81.
- Bellingham PJ & Sparrow AD** (2000). *Resprouting as a life history strategy in woody plant communities*. Oikos 89(2), 409-416.
- Berendse F** (1994). *Competition between plant populations at low and high nutrient supplies*. Oikos 71, 253-260.
- Berendse F** (1998). *Effects of dominant plant species on soils during succession in nutrient-poor ecosystems*. Biogeochemistry 42, 73-88.
- Berg B & Söderström B** (1979). *Fungal biomass and nitrogen in decomposing scot pine needle litter*. Soil Biology and Biochemistry 11, 339-341.
- Berger TW & Glatzel G** (2001). *Response of Quercus petraea seedlings to nitrogen fertilization*. Forest Ecology and Management 149, 1-14.
- Bernal MP, Sanchez-Monedero MA, Paredes C & Roig A** (1998). *Carbon mineralization from organic wastes at different composting stages during their incubation with soil*. Agriculture, Ecosystems and Environment 69, 175-189.
- Bingham FT** (1966). *Phosphorus*. Diagnostic criteria for plants and soils. (ed HD Chapman), University of California, 324-362..
- Blair JM** (1997). *Fire, N availability, and plant response in grasslands : a test of the transient maxima hypothesis*. Ecology 78(8), 2359-2368.
- Bonito GM, Coleman DC, Haines BL & Cabrera ML** (2003). *Can nitrogen budgets explain differences in soil nitrogen mineralization rates of forest stands along an elevation gradient ?* Forest Ecology and Management, 176, 563-574.
- Borken W, Muhs A & Beese F** (2002). *Application of compost in spruce forest: effects on soil respiration, basal respiration and microbial biomass*. Forest Ecology and Management 159, 49-58.
- Bouanani F** (2001). *Etude en champs et en conditions contrôlées de la minéralisation de l'azote et des modifications de l'organisation du sol, après apport de matières organiques issues de déchets urbains et agricoles*. Thèse de doctorat, Université de Provence.
- Bourg ACM** (1995). *Speciation of heavy metals in soils and groundwater and implications for their natural and provoked mobility*. Heavy metals: problems and solutions. Salomons, Förstner and Mader editors, Berlin, 19-31.
- Bowen GD** (1984). *Tree roots and the use of soil nutrients*. Nutrition of plantation forests (eds GD Bowen & EKS Nambiar), Academic Press, London, 147-180.

- Bradford GR** (1966). *Boron*. Diagnostic criteria for plants and soils (ed HD Chapman), University of California, 324-362.
- Briat JF & Lebrun M** (1999). *Plant responses to metal toxicity*. Plant Biology and Pathology 322, 43-54.
- Brockway DG** (1983). *Forest floor, soil, and vegetation responses to sludge fertilization in red and white pine plantations*. Soil Sci. Soc. Am. J. 47, 776-784.
- Buesing N & Gessner MO** (2002). *Comparison of detachment procedures for direct counts of bacteria associated with sediment particles, plant litter and epiphytic biofilms*. Aquatic Microb. Ecology 27, 29-36.

C

- Calvo L, Tarrega R & De Luis E** (2002^a). *Secondary succession after perturbations in a shrubland community*. Acta oecologica 23, 393-404.
- Calvo L, Tarrega R & De Luis E** (2002^b). *The dynamics of mediterranean shrubs species over 12 years following perturbations*. Plant Ecology 160, 25-42.
- Cambardella CA, Richard TL & Russell A** (2003). *Compost mineralization in soil as a function of composting process conditions*. European Journal of Soil Biology 39, 117-127.
- Cañellas I & San Miguel A** (1998). *Litter fall and nutrient turnover in Kermes oak (Quercus coccifera L.) shrublands in Valencia (Eastern Spain)*. Annals Sci. For. 55, 589-597.
- Caravaca F, Garcia C, Hernandez MT & Roldan A** (2002). *Aggregate stability changes after organic amendment and mycorrhizal inoculation in the afforestation of a semiarid site with Pinus halepensis*. Applied Soil Ecology 19, 199-208.
- Carreira JA & Niell FX** (1992). *Plant nutrient changes in a semi-arid Mediterranean shrubland after fire*. Journal of Vegetation Science 3, 457-466.
- Caturla RN, Raventos J, Guàrdia R & Vallejo VR** (2000). *Early post-fire regeneration dynamics of Brachypodium retusum Pers. (Beauv.) in old fields of the Valencia region (eastern Spain)*. Acta Oecologica 21(1), 1-12.
- Cemagref** (1983). *L'utilisation des déchets urbains pour la reconstitution des forêts méditerranéennes*. Convention ANRED 80.05.037, 97pp.(+34pp.).
- Chapin FSIII** (1980). *The mineral nutrition of wild plants*. Annual Review of Ecology and Systematics 11, 233-260.
- Chapin FSIII, Matson PA & Mooney HA** (2002). *Principles of terrestrial Ecosystem Ecology*. 1^{ère} ed., Springer-Verlag, Berlin, 436 pp.
- Chapman HD** (1966). *Calcium*. Diagnostic criteria for plants and soils. (ed HD Chapman), University of California, 65-85.
- Chauvet E** (1987). *Changes in the chemical composition of alder, poplar and willow leaves during decomposition in a river*. Hydrobiologia 148, 35-44.
- Chen Y, Chefetz B & Hadar Y** (1996). *Formation and properties of humic substances originating from composts*. Science of composting (eds M De Bertoldi, P Sequi, B Lemmes & T Papi), 382-393.
- Cortet J, Joffre R, Elmholt S & Krogh PH** (2003). *Increasing species and trophic diversity of mesofauna affects fungal biomass, mesofauna structure community and organic matter decomposition processes*. Biology and Fertility of Soil 37, 302-312.
- Courtois G** (2000). *Evolution de la réglementation concernant l'épandage des boues issues du traitement des eaux usées : aspects sanitaires*. Forêt méditerranéenne 21(3), 416-421.
- Criquet S** (1999). *La litière de chêne vert (Quercus ilex L.). Aspects méthodologiques, enzymologiques et microbiologiques. Etude préliminaire des relations in situ entre*

microorganismes, enzymes et facteurs environnementaux. Thèse de doctorat, Univ. Aix-Marseille III.

Criquet S, Tagger S, Vogt G & Le Petit J (2002). *Endoglucanase and β -glycosidase activities in an evergreen oak litter: annual variation and regulating factors*. *Soil Biology and Biochemistry* 34(8), 1111-1122.

Criquet S, Farnet AM, Tagger S & Le Petit J (2000). *Annual variations of phenoloxidase activities in an evergreen oak litter : influence of certain biotic and abiotic factors*. *Soil Biology and Biochemistry* 32, 1505-1513.

D

Dai J, Becquer T, Rouiller JH, Reversat G, Bernhard-Reversat F & Lavelle P (2004). *Influence of heavy metals on C and N mineralisation and microbial biomass in Zn-, Pb-, Cu-, and Cd-contaminated soils*. *Applied Soil Ecology* 25, 99-109.

Damesin C, Rambal S & Joffre R (1998). *Co-occurrence of trees with different leaf habits: a functional approach on Mediterranean oaks*. *Acta Oecologica* 19, 195-204.

Davey CB & Wollum AG (1984). *Nitrogen fixation systems in forest plantations*. *Nutrition of plantation forests* (eds GD Bowen and EKS Nambiar), Academic Press, London, 181-210.

Dell B, Hopkins AJM & Lamont BB (1986). *Introduction*. Resilience in Mediterranean-type ecosystems (eds B Dell, AJM Hopkins, BB Lamont), Dr W Junk Publishers, Dordrecht, 1-5.

De Luis M, Garcia-Cano MF, Cortina J, Raventos J, Carlos Gonzalez-Hidalgo J & Rafael-Sanchez J (2001). *Climatic trends, disturbances and short-term vegetation dynamics in a Mediterranean shrubland*. *Forest Ecology and Management* 147, 25-37.

De Nicola F, Maisto G & Alfani A (2003). *Assessment of nutritional status and trace element contamination of holm oak woodlands through analyses of leaves and surrounding soils*. *Science of the Total Environment* 311, 191-203.

Diaz-Delgado R, Lloret F, Pons X & Terradas J (2002). *Satellite evidence of decreasing resilience in Mediterranean plant communities after recurrent wildfires*. *Ecology* 83(8), 2293-2303.

Donnegan JA & Rebertus AJ (1999). *Rates and mechanisms of subalpine forest succession along an environmental gradient*. *Ecology* 80(4), 1370-1384.

Douglas DR, Saari-Nordhaus R, Despres P & Anderson Jr JM (2002). *New suppressor technology improves trace level anion analysis with carbonate-hydrogencarbonate mobile phases*. *Journal of Chromatography A* 956, 47-51.

Dutch J & Wolstenholme R (1994). *The effects of sewage sludge application to a heathland site prior to planting with Sitka spruce*. *Forest Ecology and Management* 66, 151-163.

E

Embleton TW (1966). *Magnesium*. Diagnostic criteria for plants and soils (ed HD Chapman), University of California, 225-263.

Epstein E, Keane DB, Meisinger JJ & Legg JO (1978). *Mineralization of nitrogen from sewage sludge and sludge compost*. *Journal of Environmental Quality* 7(2), 217-221.

Epstein E, Taylor JM & Chaney RL (1976). *Effect of sewage sludge and sludge compost applied to soil on some physical and chemical properties*. *Journal of Environmental Quality* 5(4), 422-426.

F

Faurie C, Ferra C, Médori P & Dévaux J (2003). *Ecologie : approche scientifique et pratique*. Lavoisier Tec & Doc, Paris.

- Feix I & Savanne D** (1998). *Evaluation des risques écotoxicologiques liés à l'épandage des déchets en agriculture*. 16^{ème} Congrès Mondial des Sciences du Sol, 20-26 août 1998, Montpellier, France, 7 pp.
- Floret C** (1988). *Méthode de mesure de la végétation pastorale*. Pastoralisme et développement, (Eds Institut agronomique méditerranéen de Montpellier (CIHEAM), France, et Institut agronomique et vétérinaire Hassan II de Rabat, Maroc), 24 mai - 9 juillet 1988, 13-21.
- Floret CH, Galan MJ, Le Floc'h E, Leprince F & Romane F** (1989). *France*. Plant Pheno-morphological Studies in Mediterranean Type Ecosystems (ed G Orshan), Kluwer Academic Publishers, Dordrecht, 9-98.
- Ferran A & Vallejo VR** (1998). *Long-term plant regeneration after wildfires in Mediterranean ecosystems of NE Spain*. Fire Management and Landscape Ecology (ed L Trabaud), International Association of Wildland Fire Fairfield, Washington, 155-166.

G

- Garcia C, Hernandez T & Costa F** (1990). *The influence of composting and maturation processes on the heavy-metal extractability from some organic wastes*. Biological Wastes 31(4), 195-219.
- Gershenzon J** (1984). *Changes in the level of plant secondary metabolites production under water and nutrient stress*. Phytochemical adaptation to stress, recent advance in phytochemistry (eds FA Loewus, BN Timmermenn & C Steelink), Plenum Press, New-York, 273-320.
- Gessner MO, Bauchowitz MA & Escutier M** (1991). *Extraction and quantification of ergosterol as a measure of fungal biomass in leaf litter*. Microbial Ecology 22, 285-291.
- Gessner MO & Chauvet E** (1994). *Importance of stream microfungi in controlling breakdown rates of leaf litter*. Ecology 75, 1807-1817.
- Gessner MO & Schmitt AL** (1996). *Use of solid phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi*. Applied Environmental Microbiology 62, 415-419.
- Gimeno-Garcia E, Andreu V & Rubio JL** (2001). *Influence of Mediterranean shrub species on soil chemical properties in typical Mediterranean environment*. Communications in Soil Science and Plant Analysis 32(11&12), 1885-1898.
- Giovannini G, Vallejo V R, Lucchesi S, Bautista S, Ciompi S & Llovet J** (2001). *Effects of land use and eventual fire on soil erodibility in dry Mediterranean conditions*. Forest Ecology and Management 147, 15-23.
- Glass ADM** (1988). *Plant nutrition: an introduction to current concepts*. Jones and Bartlett Publishers, Boston.
- Gosz JR** (1984). *Biological factors influencing nutrient supply in forest soils*. Nutrition of plantation forests (eds GD Bowen & EKS Nambiar), Academic Press, London, 119-146.
- Gouin FR & Walker JM** (1977). *Deciduous tree seedling response to nursery soil amended with composted sewage sludge*. Hort. Sci. 12(1), 45-47.
- Goyal SS, Hafez AAR & Rains DW** (1993). *Simultaneous determination of total sodium, potassium, magnesium, and calcium in plant tissues using acid digestion and ion chromatography*. Agronomy Journal 85, 1192-1197.
- Grogan P, Bruns TD & Chapin FS** (2000). *Fire effects on ecosystem nitrogen cycling in a Californian bishop pine forest*. Oecologia 122, 537-544.
- Guerrero C, Gomez I, Moral R, Mataix-Solera J, Mataix-Beneyto J & Hernandez T** (2001). *Reclamation of a burned forest soil with municipal waste compost: macronutrient dynamic and improved vegetation cover recovery*. Bioresource Technology 76, 221-227.

Gunderson L, Holling CS, Pritchard L & Peterson GD (2002). *Resilience*. Encyclopedia of Global Environmental Change (eds HA Mooney & JG Canadell), Volume 2, 530-531.

I

INRA (2001). Epanrages expérimentaux de boues sur parcelles boisées : recommandations pour la conception et le suivi de dispositifs expérimentaux. INRA Editions, version provisoire mars 2001, 50 pp.

Isidorov V & Jdanova M (2002). *Volatile organic compounds from leaves litter*. Chemosphere 48, 975-979.

H

Hernandez L, Probst A, Probst JL & Ulrich E (2003). *Heavy metal distribution in some French forest soils: evidence for atmospheric contamination*. The Science of the Total Environment 312, 191-203.

Hodgson JG, Wilson PJ, Hunt R, Grime JP & Thompson K (1999). *Allocating C-S-R plant functional types: a soft approach to a hard problem*. Oikos 85, 282-294.

Hooper DU (1998). *The role of complementarity and competition in ecosystem responses to variation in plant diversity*. Ecology 79, 704-719.

J

Jackson PE, Krol J, Heckenberg AL, Mientjes M & Staal W (1991). *Determination of total nitrogen in food, environmental and other samples by ion chromatography after Kjeldahl digestion*. Journal of Chromatography 546, 405-410.

Jensen V (1974). *Decomposition of Angiosperm tree leaf litter*. Biology of plant litter decomposition (eds CH Dickinson & GJF Pugh), Vol.1, Academic Press, London, 69-104.

Jonasson S (1983). *The point intercept method for non-destructive estimation of biomass*. Phytocoenologia 11(3), 385-388.

Jonasson S (1988). *Evaluation of the point intercept method for the estimation of plant biomass*. Oikos 52, 101-106.

Juhren MC (1966). *Ecological observations on Cistus in the Mediterranean vegetation*. Forest Science 12(4), 415-426.

K

Kabata-Pendias A & Pendias H (1992). *Trace elements in soils and plants*, 2nd ed. CRC Press LLC, USA.

Kavvadias VA, Alifragis D, Tsiontsis A, Brofas G & Stamatelos G (2001). *Litterfall, litter accumulation and litter decomposition rates in four forest ecosystems in northern Greece*. Forest Ecology and Management 144, 113-127.

Keeley JE (1986) *Resilience of Mediterranean shrub communities to fire*. Resilience in Mediterranean-type ecosystems (eds B Dell, AJM Hopkins & BB Lamont), Dr W Junk Publishers, Dordrecht, 95-112.

Khan M & Scullion J (2002). *Effects of metal (Cd, Cu, Ni, Pb or Zn) enrichment of sewage sludge on soil microorganisms and their activities*. Applied Soil Ecology 20, 145-155.

Khanna PH & Ulrich B (1984). *Soil characteristics influencing nutrient supply in forest soils*. Nutrition of plantation forests (eds GD Bowen & EKS Nambiar), Academic Press, London, 79-118

Kim CG, Power SA & Bell JNB (2003). *Effects of Cd and soil type on mineral nutrition and C partitioning in seedlings of P. sylvestris*. Water, Air, and Soil Pollution 145, 253-266.

- Korboulewsky N, Bonin G & Massiani C** (2002^a). *Biological and ecophysiological reactions of white wall rocket (*Diplotaxis erucoides* L.) grown on sewage sludge compost*. Environmental Pollution 117, 365-370.
- Korboulewsky N, Dupouyet S & Bonin G** (2002^b). *Environmental risks of applying sewage sludge compost to vineyards : carbon, heavy metals, nitrogen, and phosphorus accumulation*. Journal of Environmental Quality 31, 1522-1527.
- Kruger FJ** (1987). *Responses of plants to nutrient supply in Mediterranean-type ecosystems*. Plant responses to stress (eds JD Tenhunen *et al.*), Springer Verlag, Berlin, 415-427.
- Kunito T, Saeki K, Goto S, Hayashi H, Oyaizu H & Matsumoto S** (2001). *Copper and Zinc fractions affecting microorganisms in long-term sludge-amended soils*. Bioresource Technology 79, 135-146.
- Kwabiah AB, Palm CA, Stoskopf NC & Voroney RP** (2003). *Response of soil microbial biomass dynamics to quality of plant materials with emphasis on P availability*. Soil Biology and Biochemistry 35, 207-216.

L

- Larcher W** (1983). *Physiological plant ecology*, 2nd Ed. Springer-Verlag, Berlin.
- Le Houerou HN** (1971). *Le rôle de l'écologie végétale dans les études de mise en valeur de la région méditerranéenne*. Bulletin des Recherches Agronomiques de Gembloux, Volume extraordinaire édité à l'occasion de la semaine d'étude des problèmes méditerranéens, 68-87.
- Le Houerou HN** (1973). *Fire and vegetation in the Mediterranean basin*. Proceedings Annual Tall Timbers, Fire Ecology Conference.
- Lepart J & Escarré J** (1983). *La succession végétale, mécanismes et modèles: analyse bibliographique*. Bulletin d'Ecologie 14(3), 133-178.
- Le Scaon G** (1999). *Valorisation des boues en région PACA*. Communication orale. Colloque régional sur la valorisation des boues, 5 avril 2001, MNEP, Marseille, France.
- Lloret F & Vilà M** (1997). *Clearing of vegetation in Mediterranean garrigue: response after a wildfire*. Forest Ecology and Management 93, 227-234.
- Luo YM & Christie P** (1995). *Some short-term effects of a lime stabilised sewage sludge applied to arable and forest soils*. Irish Journal of Agriculture and Food Research 34(1), p.78.

M

- Madritch MD & Hunter MD** (2003). *Intraspecific litter diversity and nitrogen deposition affect nutrient dynamics and soil respiration*. Oecologia 136, 124-128.
- Maestre FT, Cortina J, Bautista S & Bellot J** (2003). *Does Pinus halepensis facilitate the establishment of shrubs in Mediterranean semi-arid afforestation ?* Forest Ecology and Management 176, 147-160.
- Mälkönen E, Derome J, Fritze H, Helmisaari HS, Kukkola M, Kytö, Saarsalmi A & Salemaa M.** (1999). *Compensatory fertilisation of Scots pine stands polluted by heavy metals*. Nutr. Cycl. Agroecosyst. 55, 239-268.
- Margaris NS** (1981). *Adaptive strategies in plants dominating Mediterranean-type ecosystems*. Ecosystems of the world 11. Mediterranean-type shrublands. (eds F Di Castri, DW Goodall & RL Specht), Elsevier Scientific Publishing Company, 309-315.
- Marien JN** (2000). *Quelle acceptabilité pour l'épandage de boues en forêt ?* Forêt méditerranéenne 21(3), 360-362.
- Martens R** (1995). *Current methods for measuring microbial biomass C in soil: potential and limitations*. Biology and Fertility of Soils 19, 87-99.

- Martinez F, Casermeiro MA, Morales D, Cuevas G & Walter I** (2003^b). *Effects on run-off water quantity and quality of urban organic wastes applied in a degraded semi-arid ecosystem*. The Science of the Total Environment 305, 13-21.
- Martinez F, Cuevas G, Calvo R & Walter I** (2003^a). *Biowaste effects on soil and native plants in semiarid ecosystem*. Journal of Environmental Quality 32, 472-479.
- Martin-Prével P** (1978). *Rôle des éléments minéraux chez les végétaux*. Fruits 33(7-8), 521-529.
- Martin-Prével P, Gagnard J & Gautier P** (1984). *L'analyse végétale dans le contrôle de l'alimentation des plantes tempérées et tropicales*. Techniques et Documentation, Lavoisier, Paris.
- Masson P & Andrieu M** (1996). *Simultaneous analysis of nitrogen, potassium, calcium and magnesium in digested plant samples by ion chromatography*. Analisis 24 (9-10), 380-382.
- Masson P, Hilbert G & Plenet D** (1996). *Ion chromatography methods for the simultaneous determination of mineral anions in plant sap*. Journal of Chromatography A 752, 298-303.
- McKay HM & Moffat AJ** (2001). *GB experience in the last decade: from research to practice*. Biosolids utilisation in forestry (ed INRA), International Workshop, 29-30 March 2001.
- Michaut C** (2004). *Boues, sur la voie de l'amendement*. Environnement Magazine 1626, 41-49.
- Miller HG** (1984). *Dynamics of nutrient cycling in plantation ecosystems*. Nutrition of plantation forests (eds GD Bowen & EKS Nambiar), Academic Press, London, 53-78.
- Ministère de l'Environnement** (1983). *Perspectives régionales d'utilisation agricole des boues d'épuration et matériel utilisable*. Paris, 18-26.
- Montès N, Ballini C, Bonin G & Faures J** (2004). *A comparative study of aboveground biomass of three Mediterranean species in a post-fire succession*. Acta Oecologica 25, 1-6.
- Mooney HA** (1981). *Primary production in Mediterranean-climate regions*. Ecosystems of the world 11. Mediterranean-type shrublands (eds F Di Castri, DW Goodall & RL Specht), Elsevier Scientific Publishing Company, 249-255.
- Moreno JL, Garcia C, Hernandez T & Pascual JA** (1996). *Transference of heavy metals from a calcareous soil amended with sewage-sludge compost to barley plants*. Bioresource Technology 55, 251-258.
- Moreno JL, Pérez A, Aliaga A & Hernandez T** (2003). *The ecological dose of Ni in a semiarid soil amended with sewage sludge related to the unamended soil*. Water, Air, and Soil pollution 143, 289-300.
- Moreno-Peñaranda R, Lloret F & Alcañiz JM** (2004). *Effects of sewage sludge on plant community composition in restored limestone quarries*. Restoration Ecology 12(2), 290-296.
- Mustin M** (1993). *Le compost : gestion de la matière organique* (ed François Dubusc), Cocagne, Paris, 954 pp.

N

- Navas A, Machin J & Navas B** (1999). *Use of biosolids to restore the natural vegetation cover on degraded soils in the badlands of Zaragoza (NE Spain)*. Bioresource Technology 69, 199-205.
- NF U 44-095** (2002). *Amendements organiques: Composts contenant des matières d'intérêt agronomique issues du traitement des eaux*. AFNOR, JO du 26/03/2004.

O

- Olsen SR, Cola CV, Watanabe FS & Dean LA** (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate*. USDA circular No.939.
- Orshan G** (1989). *Introduction*. Plant Pheno-morphological Studies in Mediterranean Type Ecosystems. (ed G Orshan), Kluwer Academic Publishers, Dordrecht, 1-5.

P

- Pagliali M & Antisari LV** (1993). *Influence of waste organic matter on soil micro- and macrostructure*. Bioresource Technology 43, 205-213.
- Pagliali M, Guidi G, La Marca M, Giachetti M & Lucamente G** (1981). *Effect of sewage sludge and composts on soil porosity and agregation*. Journal of Environmental Quality 10(4), 556-561.
- Papatheodorou EM, Argyropoulou MD & Stamou GP** (2004). *The effects of large- and small-scale differences in soil temperature and moisture on bacterial functional diversity and the community of bacterivorous nematodes*. Applied Soil Ecology 25, 37-49.
- Pillar VD** (1999). *On the identification of optimal plant functional types*. Journal of Vegetation Science 10.5, 631-640.
- Pillard-Landeau B** (2001). *Results of sewage sludge experimentation in Chantilly forest*. Communication du colloque sur l'Utilisation Sylvicole de Produits Résiduaire, 29-30 mars 2001, INRA, ADEME.
- Pitchel J & Anderson M** (1997). *Trace metal bioavailability in municipal solid waste and sewage sludge composts*. Bioresource Technology 60, 223-229.
- Planchais N, Renault-Miskovsky J & Vernet JL** (1977). *Les facteurs d'évolution de la végétation dans le sud de la France depuis le tardiglaciaire d'après l'analyse pollinique et les charbons de bois*. Approche écologique de l'homme fossile, supplément au Bulletin de l'Association Française d'Etude du Quaternaire, 323-327.
- Planquart P, Bonin G, Prone A & Massiani C** (1999). *Distribution, movement and plant availability of trace metals in soils amended with sewage sludge composts: application to low metal loadings*. The Science of the Total Environment 241, 161-179.
- Porter KG & Feig YS** (1980). *The use of DAPI for identifying and counting aquatic microflora*. Limnol. Oceanogr. 25, 943-948.

Q

- Querejeta JI, Roldan A, Albaladejo J & Castillo V** (2001). *Soil water availability improved by site preparation in a Pinus halepensis afforestation under semiarid climate*. Forest Ecology and Management 149, 115-128.
- Quézel P** (1981). *The study of plant grouping in the countries surrounding the mediterranean: some methodological aspects*. Ecosystems of the world 11. Mediterranean-type shrublands (eds F Di Castri, DW Goodall & RL Specht), Elsevier Scientific Publishing Company, 87-94.
- Quézel P & Médail F** (2003). *Ecologie et biogéographie des forêts du bassin méditerranéen*. Elsevier, Environmental series, Paris, 570 pp.
- Quispel A** (1983). *Dinitrogen-fixing symbioses with Legumes, Non-Legume Angiosperms and associative symbioses*. Inorganic Plant Nutrition, Encyclopedia of Plant Physiology. (eds A Läuchli & RL Bielecki), New Series, Vol.15A, Springer-Verlag, Berlin, 286-329.

R

- Ramade F** (2002). *Dictionnaire encyclopédique de l'Ecologie et des Sciences de l'Environnement*, 2ème édition, Dunod, Paris, 1075 pp.

- Ranger J & Turpault MP** (1999). *Input-output nutrient budgets as a diagnostic tool for sustainable forest management*. Forest Ecology and Management 122, 139-154.
- Rapp M, Santa Regina I, Rico M & Gallego HA** (1999). *Biomass, nutrient content, litterfall and nutrient return to the soil in Mediterranean oak forests*. Forest Ecology and Management 119, 39-49.
- Ripert C** (2000^a). *Utilisation de composts d'ordures ménagères en reboisements méditerranéens*. Forêt méditerranéenne 21(3), 374-380.
- Ripert C** (2000^b). *Reboisement méditerranéen avec des boues résiduaires urbaines : Essai de Carpiagne (1982-1987)*. Forêt méditerranéenne 21(3), 387-390.
- Ros M, Hernandez MT & Garcia C** (2003). *Soil microbial activity after restoration of a semiarid soil by organic amendments*. Soil Biology and Biochemistry 35, 463-469.
- Rundel PW** (1988). *Leaf structure and nutrition in Mediterranean-climate sclerophylls*. Mediterranean-type Ecosystems (ed RL Specht), Kluwer Academic publishers, 157-167.
- Russo VM & Karmarkar SV** (1998). *Water extraction of plant tissues for analysis by ion chromatography*. Communications on Soil Science and Plant Analysis 29(3&4), 245-253.

S

- Saari-Nordhaus R & Anderson Jr JM** (2002). *Recent advances in ion chromatography suppressor improve anion separation and detection*. Journal of Chromatography A 956, 15-22.
- Sands R** (1984). *Environmental aspects of plantation management*. Nutrition of plantation forests (GD Bowen & EKS Nambiar), Academic Press, London, 413-438.
- Santa Regina I, Leonardi S & Rapp M** (2001). *Foliar nutrient dynamics and nutrient-use efficiency in Castanea sativa coppice stands of southern Europe*. Forestry 74(1), 3-10.
- Sawhney BL, Bugbee GJ & Stilwell DE** (1995). *Heavy metal leachability as affected by pH of compost-amended growth medium used in container-grown rhododendrons*. Compost Sci. Util. 3(2), 64-73.
- Scarascia-Mugnozza G, Oswald H, Piussi P & Radoglou K** (2000). *Forests of the Mediterranean region : gaps in knowledge and research needs*. Forest Ecology and Management 132, 97-109.
- Schallenberg M, Kalff J & Rasmussen JB** (1989). *Solutions to problems in enumerating sediment bacteria by direct counts*. Applied Environmental Microbiology 55, 1214-1219.
- Schulze ED** (1995). *Flux control at the ecosystem level*. Tree 10(1), 40-43.
- Senesi N** (1989). *Composted materials as organic fertilizers*. The Science of the Total Environment 81/82, 521-542.
- Sims JT & Kline JS** (1991). *Chemical fractionation and plant uptake of heavy metals in soils amended with co-composted sewage sludge*. Journal of Environmental Quality 20, 387-395.
- Shi W, Bischoff M, Turco R & Konopka A** (2002). *Long term effects of chromium and lead upon the activity of soil microbial communities*. Applied Soil Ecology 21(2), 169-177.
- Stevens TS** (2002). *The membrane suppressor: a historical perspective*. Journal of Chromatography A 956, 43-46.

T

- Tenhunen JD, Meister HP, Caldwell MM & Lange OL** (1984). *Environmental constraints on productivity of the Mediterranean sclerophyll shrub Quercus coccifera*. Workshop agroecology, CIHEAM, Paris, 33-53.

Thirukkumaran CM & Parkinson D (2002). *Microbial activity, nutrient dynamics and litter decomposition in a Canadian Rocky Mountain pine forest as affected by N and P fertilizers*. *Forest Ecology and Management* 159, 187-201.

Tilman GD (1984). *Plant dominance along an experimental nutrient gradient*. *Ecology* 65(5), 1445-1453.

Toutain E (1987). *Les litières: siège de systèmes interactifs et moteurs de ces interactions*. *Ecol. Biol. Sol* 24 (3), 231-242

Trabaud L (1987). *Dynamics after fire of sclerophyllous plant communities in the mediterranean basin*. *Ecologia mediterranea* XIII, 25-38.

U

Urbas P & Zobel K (2000). *Adaptive and inevitable morphological plasticity of three herbaceous species in a multi-species community: field experiment with manipulated nutrients and light*. *Acta Oecologica* 21(2), 139-147.

V

Van Breemen N & Finzi AC (1998). *Plant-soil interactions: ecological aspects and evolutionary implications*. *Biogeochemistry* 42, 1-19.

Van Den Driessche R (1984). *Nutrient storage, retranslocation and relationship of stress to nutrition*. *Nutrition of plantation forests* (eds GD Bowen & EKS Nambiar), Academic Press, London, 181-210.

Vernet JL (1973). *Etude sur l'histoire de la végétation du sud-est de la France au quaternaire, d'après les charbons de bois principalement*. *Paléobiologie Continentale* 4, 1-90.

Ville de Marseille (1988). *Reforestation du plateau de Carpiagne*. Etude d'impact, dossier complémentaire No.3.

W

Wardle DA, Barker GM, Bonner KI & Nicholson KS (1998). *Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems?* *Journal of Ecology* 86, 405-420.

Wardle DA, Yeates GW, Nicholson KS, Bonner KI & Watson RN (1999). *Response of soil microbial biomass dynamics, activity and plant litter decomposition to agricultural intensification over a seven-year period*. *Soil Biology and Biochemistry* 31, 1707-1720.

Welander NT & Ottosson B (2000). *The influence of low light, drought and fertilization on transpiration and growth in young seedlings of Quercus robur L*. *Forest Ecology and Management* 127, 139-151.

Westman WE (1986). *Resilience: concepts and measures*. *Resilience in Mediterranean-type ecosystems* (eds B Dell, AJM Hopkins & BB Lamont), Dr W Junk Publishers, Dordrecht, 5-20.

White PS & Jentsch A (2001). *The search for generality in studies of disturbance and ecosystem dynamics*. *Progress in Botany* 62, 399-449.

Wisniewski L & Dickinson NM (2003). *Toxicity of copper to Quercus robur (English Oak) seedlings from a copper-rich soil*. *Environmental and Experimental Botany* 50, 99-107.

Z

Zahrer L & Lawrence DG (2001). *Applying compost: benefits and needs*. Seminar proceedings, 22-23 Novembre 2001, Bruxelles, 1-8.

Zar JH (1984). *Biostatistical analysis*, 2nd ed. Prentice-Hall International, U.K.

Zasoski RJ & Edmonds RL (1986). *Water quality in relation to sludge and wastewater applications to forest land*. The forest alternative for treatment and utilization of municipal and industrial wastes (eds DW Cole, CL Henry & WL Nutter), University of Washington Press, Seattle and London, 100-109.

Zwonitzer JC, Pierzynski GM & Hettiarachi GM (2003). *Effects of phosphorus additions on lead, cadmium, and zinc bioavailabilities in a metal-contaminated soil*. *Water, Air, and Soil pollution* 143, 193-209.

ANNEXE**DOSAGE DE L'AZOTE ET DES CATIONS DANS LE MATERIEL VEGETAL
PAR CHROMATOGRAPHIE IONIQUE**

Introduction

Actuellement, l'analyse des éléments totaux dans les végétaux ne fait l'objet d'aucune norme.

Le processus d'analyse comporte deux phases :

- la minéralisation, qui transforme la matière organique des échantillons en éléments simples et détectables en solution
- le dosage, qui utilise les propriétés particulières à chaque élément pour estimer leur concentration

Les méthodes de dosage usuelles sont diverses et particulières à chaque élément (ICP-MS, spectrométrie d'émission ou d'absorption, N-Kehldahl), ce qui augmente le temps de traitement, multiplie les manipulations et nécessite une quantité importante d'échantillon végétal.

Le dosage de l'azote et des cations du matériel végétal par chromatographie ionique fait intervenir une seule analyse pluriéléments, ce qui diminue les coûts et le temps d'analyse par rapport à la spectrométrie d'absorption atomique et la spectrométrie d'induction plasma (Jackson *et al.* 1991 ; Goyal *et al.* 1993). De plus, la grande sensibilité de détection permet de travailler sur de très faibles quantités d'échantillon (Masson *et al.* 1996). Enfin, cette méthode semble donner des résultats aussi fiables que les méthodes traditionnelles pour le dosage des cations (Basta & Tabatabai 1985).

Cependant, cette méthode est encore peu développée pour les analyses végétales, et reste surtout utilisée pour le dosage des éléments dans les eaux. En effet, les solutions injectées pour analyse doivent être peu concentrées en composés organiques, acides forts et sels métalliques solubles autres que alcalins ou alcalino-terreux (Basta & Tabatabai 1985), ce qui implique d'adapter l'étape de minéralisation des échantillons à ce type de dosage.

A. MINERALISATION

Des solutions diverses ont été testées pour trouver un processus de minéralisation adapté au dosage des éléments par chromatographie ionique dans les poudres végétales. Basta & Tabatabai (1985) recommandent la calcination, qui permet la minéralisation complète de l'échantillon sans ajout de sels ou d'acide concentré. Cependant, le chauffage à 550°C préconisé risque de provoquer des pertes en azote par volatilisation et limite cette méthode au dosage des cations. Certains auteurs préconisent donc la combustion en présence d'oxygène dans une enceinte fermée. D'autres proposent une extraction à l'eau ultrapure (Russo &

Karmarkar 1998). Mais la digestion acide à chaud reste la méthode de référence pour minéraliser le matériel végétal (Goyal *et al.* 1993).

Méthode de minéralisation de référence

Masson *et al* (1996) proposent une méthode de minéralisation par voie acide compatible avec le dosage de NH_4^+ , K^+ , Mg^{2+} et Ca^{2+} par chromatographie ionique. Ils laissent reposer 250 mg de poudre végétale dans 5 ml d'acide sulfurique concentré et 5 ml d'eau oxygénée pendant une nuit, puis chauffent le mélange à 400°C pendant quatre heures.

Test de la méthode de minéralisation

L'estimation de la variabilité inter-échantillon liée au matériel de minéralisation, ainsi que le rendement de la méthode de minéralisation sont effectués.

Le rendement est calculé grâce à l'utilisation de matériel végétal de référence (branches et feuilles de buisson), dont les teneurs en éléments sont connues et certifiées (Tableau 1)

TABLEAU 1

TENEURS DE REFERENCE DANS LA POUDRE VEGETALE UTILISEE POUR ANALYSE

| N (% MS) | K (% MS) | Mg (% MS) | Ca (% MS) |
|------------|-------------|---------------|-------------|
| 1,2 (0,02) | 0,85 (0,03) | 0,287 (0,011) | 2,22 (0,07) |

Variabilité inter-échantillon

- On minéralise 12 échantillons de 250 mg de poudre de référence selon la méthode décrite par Masson *et al* (1996).
- Les solutions à minéraliser sont placées dans deux blocs à résistance chauffante. Au dessus de chaque matras est disposé un système de refroidissement à eau. Ce dernier évite la perte d'éléments par vaporisation pendant le chauffage.

On compare les valeurs obtenues pour chaque élément entre elles, à l'exclusion du matras 3 qui semble avoir été contaminé (Tableau 2). Le chauffage des matras apparaît homogène entre les deux blocs. La variabilité maximale de la méthode de minéralisation dans sa globalité (manipulations, minéralisation, dosage) est autour de 10 %.

TABLEAU 2
VARIABILITE INTER-ECHANTILLON

| | N (% MS) | K (% MS) | Mg (% MS) | Ca (% MS) |
|---|-----------|-----------|-----------|-----------|
| Matras 1 | 1,02 | 0,89 | 0,329 | 2,88 |
| Matras 2 | 1,04 | 0,86 | 0,335 | 2,90 |
| Matras 4 | 1,03 | 0,89 | 0,338 | 2,82 |
| Matras 5 | 1,08 | 0,85 | 0,309 | 2,89 |
| Matras 6 | 1,06 | 0,88 | 0,301 | 2,76 |
| Matras 7 | 0,98 | 0,91 | 0,325 | 2,90 |
| Matras 8 | 1,03 | 0,97 | 0,337 | 2,82 |
| Matras 9 | 1,02 | 0,89 | 0,324 | 2,66 |
| Matras 10 | 1,06 | 0,89 | 0,324 | 2,66 |
| Matras 11 | 1,06 | 0,90 | 0,314 | 2,67 |
| Matras 12 | 0,99 | 0,88 | 0,323 | 2,66 |
| Moyenne | 1,03 | 0,89 | 0,323 | 2,78 |
| Erreur standard | 0,01 | 0,01 | 0,004 | 0,03 |
| Variabilité maximale inter-échantillon | 10 | 13 | 11 | 10 |

variabilité maximale = (valeur maximale - valeur minimale) / moyenne x 100

Rendement de la méthode de référence

- Les teneurs obtenues pour les onze échantillons cités plus haut sont comparées aux valeurs de référence certifiées pour l'azote, le potassium, le magnésium et le calcium (Tableau 3).
- On ne récupère que 86 % de l'azote contenu dans la poudre végétale de référence. Ceci peut être expliqué par une volatilisation sous forme N₂ pendant le chauffage (température, durée trop élevées), ou bien par une minéralisation incomplète (température trop faible, durée trop courte).
- On obtient des quantités en calcium et magnésium plus importantes que ce qu'on attendait. L'explication la plus plausible est une erreur d'estimation des concentrations pour ces éléments dans les étalons de la chromatographie ionique. En effet, ces ions sont utilisés sous forme hydratée pour la préparation des solutions mères. Peut-être la quantité d'eau dans les sels est-elle sous-estimée, ce qui induirait une surestimation de la quantité d'ion utilisée pour la fabrication de la solution mère au moment de la pesée. Donc une surestimation des concentrations en calcium et en magnésium dans les solutions étalons.

TABLEAU 3

EFFICACITE DE LA METHODE DE MASSON *ET AL* (1996)

| | N (% MS) | K (% MS) | Mg (% MS) | Ca (% MS) |
|-------------------------|-----------|------------|------------|------------|
| Moyenne (n=11) | 1,03 | 0,89 | 0,323 | 2,78 |
| Valeur certifiée | 1,20 | 0,85 | 0,287 | 2,22 |
| Efficacité (%) | 86 | 105 | 113 | 125 |

Amélioration du rendement

Nous allons tenter d'améliorer le rendement pour l'azote en faisant varier les paramètres clé de la minéralisation :

- température de chauffage
- durée de chauffage

Optimisation de la température de chauffage

- On minéralise 12 échantillons de 250 mg de poudre de référence pendant 4 heures
- Quatre lots de trois échantillons correspondent à chaque température testée : 375, 400, 425 et 450°C (Figure 1).
- La température de minéralisation influence peu le rendement de la méthode. Cependant, il semble que les teneurs en azote augmentent très légèrement avec celle-ci. Nous allons donc tester l'effet de la durée de minéralisation à 450°C.

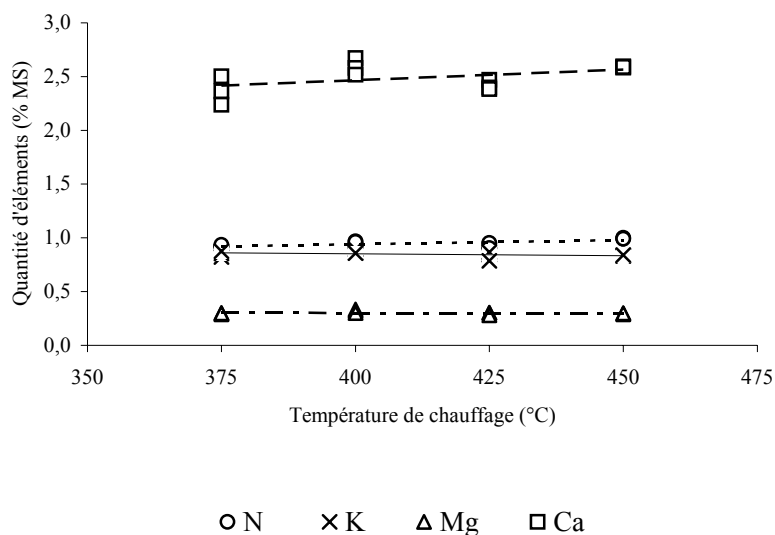


FIGURE 1

TEST DES DIFFERENTES TEMPERATURES DE MINERALISATION

Optimisation du temps de chauffage

- On minéralise 12 échantillons de 250 mg de poudre de référence à 450°C.
- Six lots de deux échantillons correspondent à chaque durée testée : 2, 3, 4, 5, 6 et 7 heures (Figure 2).
- Il apparaît que la durée du chauffage n'influence pratiquement pas le rendement de la minéralisation à 450°C.
- Par contre, lorsqu'on calcule l'efficacité globale de la minéralisation à 450°C (Tableau 4), on s'aperçoit qu'elle est bien inférieure à celle obtenue à 400°C pour l'azote et le potassium.
- On répète donc les tests de la durée de chauffage à 400°C (Figure 3).

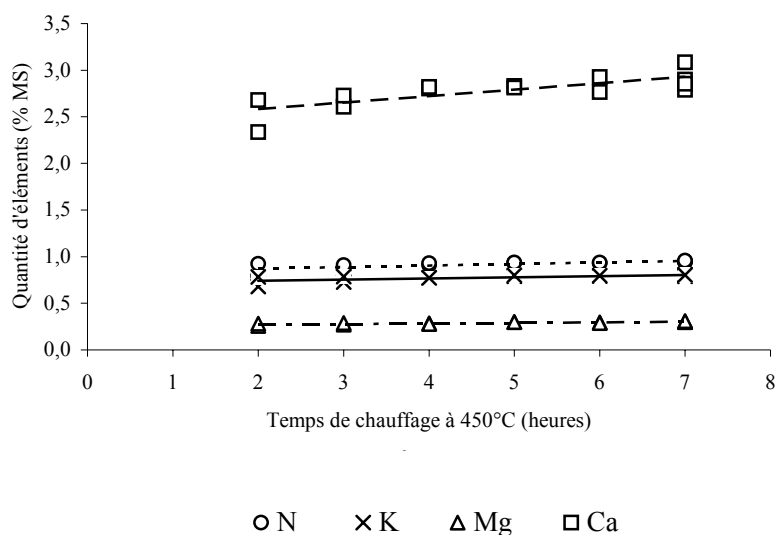


FIGURE 2
TEST DES DIFFERENTES DUREES DE MINERALISATION A 450°C

TABLEAU 4
COMPARAISON DE L'EFFICACITE DE LA MINERALISATION A 450°C ET A 400°C

| | N | K | Mg | Ca |
|-------------------------------|----------|----------|-----------|-----------|
| Efficacité à 450°C (%) | 77 | 91 | 101 | 125 |
| Efficacité à 400°C (%) | 86 | 105 | 113 | 125 |

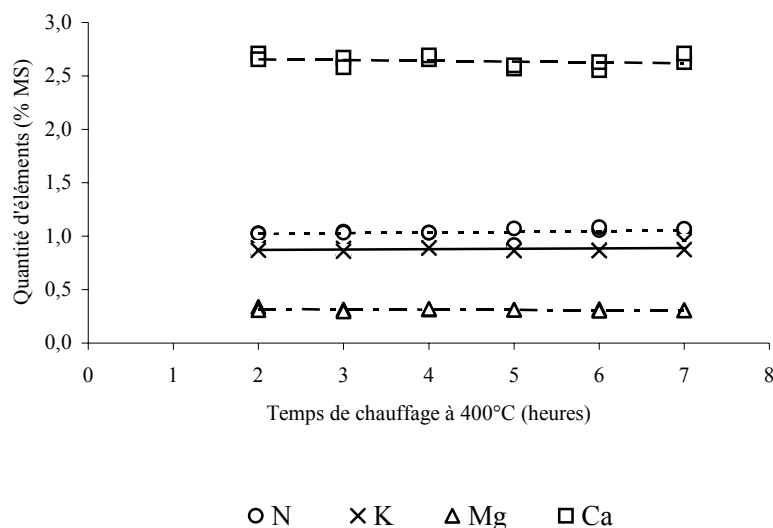


FIGURE 3

TEST DES DIFFERENTES DUREES DE MINERALISATION A 400°C

- La durée du chauffage n'influence pas le rendement de la minéralisation.
- On choisit donc une durée de minéralisation de trois heures au lieu de quatre heures, afin de diminuer le temps de traitement des échantillons.

Méthode de minéralisation optimisée

- 250 mg de poudre végétale sont mis à reposer une nuit dans un mélange de 5 mL de H_2SO_4 et 5 mL de H_2O_2 , puis chauffés pendant 3 heures à 400°C.

B. DOSAGE

- L'appareil utilisé est de type Dionex DX-120. La colonne cations est la Ionpack CS-16. Cette chromatographie ionique est équipée d'un suppresseur (Douglas *et al.* 2002. ; Saari-Nordhaus *et al.* 2002 ; Stevens 2002).
- Tous les réactifs sont de qualité analytique. L'eau ultrapure est obtenue par filtration d'eau déminéralisée sur cartouche Millipore.

Calibration de la chromatographie ionique

Dilution et filtration des minéralisats

- Les solutions de minéralisation sont diluées au 1/500^{ème} afin d'obtenir un pH compris entre 0 et 1 satisfaisant pour le dosage par chromatographie ionique équipée de la CS 16 ($0 < \text{pH} < 14$).
- Elles sont ensuite filtrées à 0,45 μm .

Force de l'éluant

- L'éluant utilisé est une solution d'acide méthane-sulfonique à 26mM. Cette concentration permet une séparation correcte des pics correspondant à chacun des ions dosés. L'analyse dure 30 minutes par échantillon.

Etalonnage

- Les étalons utilisés sont des solutions multi-cationiques (Tableau 5)

TABLEAU 5
CONCENTRATION DES SIX ETALONS

| | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Standard 5 | Standard 6 |
|------------------------------------|------------|------------|------------|------------|------------|------------|
| NH ₄ ⁺ (ppm) | 0,0125 | 0,05 | 0,2 | 0,8 | 2 | 5 |
| K ⁺ (ppm) | 0,025 | 0,1 | 0,4 | 1,6 | 4 | 10 |
| Mg ²⁺ (ppm) | 0,0125 | 0,05 | 0,2 | 0,8 | 2 | 5 |
| Ca ²⁺ (ppm) | 0,05 | 0,2 | 0,8 | 3,2 | 8 | 20 |

- La calibration est estimée correcte lorsque le coefficient de corrélation linéaire est supérieur à 0,99 et le pourcentage de dispersion des points étalons autour de la droite de calibration inférieur à 5%.
- Les valeurs limites en % MS de poudre végétale correspondant à la gamme d'étalonnage sont indiquées dans le Tableau 6.

TABLEAU 6
VALEURS LIMITES DOSEES DANS LE VEGETAL

| | N (% MS) | K (% MS) | Mg (% MS) | Ca (% MS) |
|-----------------|----------|----------|-----------|-----------|
| Valeur minimale | 0,19 | 0,5 | 0,25 | 1 |
| Valeur maximale | 78 | 100 | 100 | 100 |

Variabilité analytique de la méthode de dosage

- Des réplicats issus de la même solution de minéralisation sont analysés pour évaluer la variabilité due à la méthode de dosage (Tableau 7).

- La variabilité analytique est très faible pour l'azote et le potassium (1%). Elle augmente pour le magnésium, tout en restant acceptable (4%), et pour le calcium, pour lequel elle atteint 8 %.

TABLEAU 7
VARIABILITE ANALYTIQUE

| | N (% MS) | K (% MS) | Mg (% MS) | Ca (% MS) |
|--------------------------------|----------------|----------------|----------------|----------------|
| réplicat n°1 | 0,99 | 0,88 | 0,32 | 2,66 |
| réplicat n°1 | 1,00 | 0,87 | 0,32 | 2,62 |
| réplicat n°1 | 1,01 | 0,88 | 0,33 | 2,74 |
| moyenne | 1,00 | 0,88 | 0,32 | 2,67 |
| écart maximal (%) | 1,69 | 0,64 | 2,40 | 4,42 |
| réplicat n°2 | 1,07 | 0,87 | 0,30 | 2,71 |
| réplicat n°2 | 1,08 | 0,87 | 0,32 | 2,46 |
| réplicat n°2 | 1,08 | 0,85 | 0,29 | 2,75 |
| moyenne | 1,08 | 0,86 | 0,30 | 2,64 |
| écart maximal (%) | 0,62 | 2,32 | 7,56 | 10,98 |
| réplicat n°3 | 0,91 | 0,787 | 0,281 | 2,383 |
| réplicat n°3 | 0,93 | 0,794 | 0,283 | 2,498 |
| réplicat n°3 | 0,93 | 0,807 | 0,277 | 2,352 |
| moyenne | 0,92 | 0,80 | 0,28 | 2,41 |
| écart maximal (%) | 2,21 | 0,00 | 2,07 | 6,08 |
| réplicat n°4 | 0,943 | 0,789 | 0,297 | 2,789 |
| réplicat n°4 | 0,951 | 0,797 | 0,298 | 3,086 |
| réplicat n°4 | 0,957 | 0,803 | 0,310 | 2,854 |
| moyenne | 0,95 | 0,80 | 0,30 | 2,91 |
| écart maximal (%) | 1,42 | 1,73 | 4,31 | 10,22 |
| écart maximal moyen (%) | 1 (0,3) | 1 (0,5) | 4 (1,3) | 8 (1,6) |

écart maximal = (valeur maximale - valeur minimale) / moyenne x 100
(erreur standard)