HABILITATION à DIRIGER LES RECHERCHES

Ecole doctorale SIBAGHE

Comment concilier l'étude des processus régulant la biodisponibilité des éléments nutritifs

et

l'innovation agro-écologique et industrielle

Soutenance publique le 10 avril 2015 devant le jury composé de :

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Présentée par :

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A mes enfants, mes petites lumières…

Avant propos

Ce dossier est déposé pour candidater à l'Habilitation à Diriger les Recherches. Dans ce document, je présente mes travaux de recherches, mes activités d'enseignement et d'animation collectives car il est ne m'est pas possible de décorréler arbitrairement les deux. Les travaux d'enseignement et de recherche sont le bilan de 9 années d'activité. C'est une durée suffisamment longue qui permet de dégager des constantes de profil et de mûrir une réflexion stratégique.

Le document présent est composé de 5 parties :

- présentation et curriculum vitae (page 7) ;
- présentation de cinq publications marquantes (page 13) ;
- synthèse des travaux. Après une brève présentation de ma problématique générale et de son contexte scientifique, je décris ma démarche, les concepts et mes principaux résultats (page 19) ;
- réflexion sur les activités de recherche et d'enseignement en lien avec la partie précédente (page 43) ;
- projet scientifique (page 46)

Mon dossier est complété par des annexes décrivant les encadrements doctoraux et post doctoraux.

Remerciements

Je voudrai remercier les différentes personnes de mon jury ayant accepté de rapporter et d'examiner ce document et d'être présentes à la soutenance.

Je voudrai également remercier mon institution d'appartenance, Montpellier SupAgro de m'avoir recrutée puis soutenue par des financements de projets de recherche mais également en créant un environnement de travail agréable. Je tiens à souligner également l'importance de mon UMR d'affectation Eco&Sols et l'ensemble de son personnel dans la construction de mon projet de recherche. Je tiens à souligner la bienveillance de la direction de l'UMR Eco&Sols, Jean Luc Chotte, Philippe Hinsinger, Jean Michel Harmand et antérieurement Benoit Jaillard d'avoir pris en compte les contraintes des enseignants chercheurs afin de les inclure dans la vie scientifique de l'unité.

Je suis particulièrement reconnaissante envers Philippe Hinsinger et Claude Plassard de m'avoir permis de développer mon projet de recherche et soutenue de différentes façons. Merci également à Yves Dudal de m'avoir lancée sur les activités enzymatiques du sol, et présentée à Emmanuel Belamie avec qui j'ai beaucoup apprécié de travailler. Je salue les conseils et avis de Isabelle Bertrand pour la rédaction de ce rapport et la remercie de ses conseils et encouragements. Merci également à Simon Boudsocq de m'avoir écoutée et donné son avis d'écologue sur mon projet de recherche.

J'aimerai souligner la qualité de l'équipe pédagogique de Montpelluer SupAgro et en particulier Brigitte Brunel et Claire Marsden de l'axe 3, ainsi que Jean Louis Aznar, technicien de recherche et de formation. Je voudrai également remercier mes collègues enseignants et non enseignants du département MPRS et des autres départements. J'adresse un petit clin d'œil spécial à Anne Pellegrino, Aurélie Metay et Elena Kazakou, enseignantes chercheurs à Montpellier SupAgro. Je salue également Patrick Hermann qui m'a transmis le flambeau à Montpellier SupAgro. Je n'oublie mes collègues universitaires et en particulier Bastien Mérigot, Jehan Hervé Lignot et Catherine Moulia pour leurs échanges, et qualités humaines.

Un grand merci aux étudiants de doctorat et post doctorat que j'ai co encadré. J'ai beaucoup appris à leur contact, merci pour votre confiance, et qui malgré les journées parfois longues et difficiles, n'ont jamais failli et ont fait avancer nos thématiques de recherches. Merci à Nicolas, Patrice, Christophe et Clémence. Merci également aux étudiants de M1 et M2 et CDD qui ont contribué à ce projet de recherche (Ando, Claire, Alix, Arshad, Pierre, Nicolas, Simon, Jennifer, Justine). Je n'oublie pas les étudiants de M1, M2 de Montpellier SupAgro et du master Ecosystèmes que j'ai pu encadrer ou accompagner au cours de ces dernières années

… et merci à tous ceux qui ont contribué à leur manière à ce travail.

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Présentation du candidat

CANDIDAT : EDITH LE CADRE

Nationalité Française
38 ans, née le 13 août 1976
Trois enfants (naissances en 2008, 2010 et 2013).
Maître de conférences Montpellier SupAgro, CNECA2. Service d'enseignement théorique : 192 équivalent TD (éqTD)

Congé parental entre 2010 et 2013 (temps partiel 80% soit service d'enseignement théorique de 154h éq. TD).

Adresse institutionnelle :
UMR 1222 Ecologie Fonctionnelle & Biogéochimie des Sols & Agroécosystèmes (Eco&Sols) Montpellier SupAgro - INRA - IRD - CIRAD
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DIPLOMES

2005 – présent : Enseignant Chercheur en géochimie, section CNECA 2, département Milieux Productions, Ressources, Systèmes (MPRS), Montpellier SupAgro, France, affectée à l'UMR Eco&Sols.

2004-2005 : Ingénieur Recherche – Développement TIMAC agro International, responsable Productions végétales. Saint Malo, France.

2004 : Qualification CNU 37 (Météorologie, océanographie, physique de l'environnement)

2001-2004 : Doctorante à l'UMR Environnement et Grandes Cultures, Thiverval Grignon. Modélisation de la volatilisation de l'azote ammoniacal en interaction avec les processus physico chimiques et biologiques du sol après application d'engrais de synthèse, Spécialité Sciences du sol et Bioclimatologie, Ecole Doctorale ABIES. Bourse CIFRE Grande Paroisse SA, Paris la Défense, France.

2000 : M2 en sciences agronomiques. Institut National Polytechnique de Lorraine, Nancy, France.

1999 : M1 Biologie cellulaire et physiologie végétale. Université de Rennes 1, France.

PROJETS DE RECHERCHE ET FINANCEMENTS

Les différents projets que j'ai obtenu pour financer mes travaux de recherche et étudiants sont de type collaborations de recherche avec un partenaire privé et de type ANR ou appels d'offre interne. Certains travaux ont bénéficié de fonds non fléchés par un projet spécifique et seront indiqués "autre" dans le Tableau 1.

Projet 1. 2011 – Présent. Projet ANR blanc UnlockP. Coordinateur Claude Plassard. **Participant** task 5.2 et task 4 (330 k€, dont 174 k€ pour Eco&Sols)
Projet 2. 2011 – 2014. TIMAC Agro INTERNATIONAL Projet CIFRE Nutrition P (CONFIDENTIEL). **Co porteur** (124 k€ dont 55 k€ pour Eco&Sols)
Projet 3. 2012 – 2013. TIMAC Agro INTERNATIONAL Projet Qsol. **Porteur** (52 k€)

Projet 4. 2011 – 2012. IMERYS Ceramics France. Feldspath et nutrition potassique des plantes. **Co porteur** (59 k€)

Projet 5. 2010 – 2013. Projet AIDY (Analyse Intégrée des DYnamiques de conversion vers la viticulture biologique). **Participant** (total 979 k€)

Projet 6. 2011-2012 AO interne UMR Eco&Sols : Variations de la disponibilité du phosphore dans les agro-écosystèmes avec associations de plantes fixatrices et non fixatrices d'azote. Coordinateur Louis Mareschal. **Participant** (total 3,5 k€).

Projet 7. 2010-2011. AO interne UMR Eco&sols : Qualité des sols viticoles. **Co porteur** (12 k€).

Projet 8. 2010-2010. Projet Encapsulation protéines (CONFIDENTIEL). Co porteur (15 k€).

Projet 9. 2008-2012 ANR Systerra Perfcom Peuplements complexes performants en agriculture bas intrants – Interaction multitrophiques et facilitation intergénotypiques. Coordinateur Philippe Hinsinger. **Participant** (992 k€).

ENCADREMENT DE LA RECHERCHE

Depuis 2005, j'ai encadré ou co-encadré 13 étudiants dont 3 étudiants en thèse de doctorat et 1 post doctorat. Dans le Tableau 1, je résume les principales données concernant ces encadrements.

Tableau 1 : Liste des étudiants encadrés depuis 2005 avec présentation de leur valorisation (articles dans des revues internationales à comité de lecture ou brevets), et insertion professionnelle. Le nombre d'articles en premier auteur des étudiants est signalé entre parenthèses.

Nom Prénom Années et durée (mois)	Projet	Niveau	% encadrement	Valorisation (dont 1^{er} auteur)	Titre	Insertion professionnelle
Post Doctorat 1 personne						
Salome C. 2013 (12)	Projet 3	Post doctorat	100	1 (1)	Identification et analyse des indicateurs de qualité des sols pour une gestion durable des écosystèmes viticoles	CDD contrat privé, Suisse
Doctorat 4 étudiants						
Coll P. 2011 (36)	Projet 5 Projet 7	Doctorat	50	4 (2)	Qualité des sols viticoles en Languedoc Roussillon, effets des pratiques agricoles	CDI Laboratoire Rière
Lardo E. 2011 (12)	Projet 5 Projet 7	Doctorat	50	2 (1)	Study of carbon cycle and environmental sustainability in the vineyard systems for quality wine production	CDD Université Basilicata (Italie)
Devau N. 2010 (36)	Projet 9	Doctorat	33	5 (4)	Processus rhizosphériques déterminant la disponibilité du phosphore : apport de la modélisation mécaniste géochimique	Chercheur (BRGM)
Trouillefou C. 2014 (36)	Projet 2	Doctorat	33	3 (3)	Application de la biocatalyse supportée pour la nutrition phosphatée des plantes à partir de phytate.	En recherche de post doctorat
M2 : 5 étudiants						
Vidal A. 2013 (5)	Projet 1	M2	100	0	Influence des facteurs d'un sol mycorhizosphérique sur les phytases d'*Aspergillus niger* et de *Debaryomyces castelli*	Thèse UPMC (UMR Metis)
Gorbyk M. 2013 (5)	Projet 3	M2	50	-	Comment concilier pratiques viticoles et qualité des sols ? Analyse des pratiques à partir d'enquêtes	Inconnu
Trouillefou C. 2010 (6)	Projet 8	M2	33	1 (1)	Encapsulation et fonctionnement d'enzymes dans des matériaux mésoporeux	Thèse
Coll P. 2008 (6)	autre	M2	50		Sélection d'indicateurs physico chimiques et biologiques de la qualité des sols Cas de l'enherbement de la vigne	Thèse, CDI
Devau N. 2007 (6)	autre	M2	50		Modélisation de l'influence du pH de la rhizosphère sur la biodisponibilité du phosphore	Thèse, chercheur BRGM
CDD ingénieur d'étude : 2 personnes						
Julbe J.	Projet 2	CDD	100	-		
Simon B. 2012 (6)	Projet 4	CDD	50	0	Feldspath et nutrition potassique des plantes	CDD contrat privé (Cameroun)
M1 : 3 étudiants						
Collet P. 2007 (3)	autre	M1	33	0	Synthèse d'un micro-environnement racinaire pour étudier l'hétérogénéité du sol	Thèse
Moret N. 2007 (3)	autre	M1	50	0	Amélioration de la prise en compte du temps de latence de la modélisation mécaniste de la nitrification	Inconnu
Radianelson A. 2006 (2)	autre	M1	100	0	Etude bibliographique de la modélisation de l'urée	M2, thèse, post doctorat IRRI

ACTIVITES D'ENSEIGNEMENTS

Mon activité d'enseignement depuis mon recrutement en 2005 est d'en moyenne 221h équivalents TD (éqTD), et a atteint 240h éqTD en 2012-2013. J'ai été en congé parental (temps partiel 80%) entre 2010 et 2013. Depuis 2012, je suis co-responsable du parcours M2 recherche « Ecosystèmes » du master Biologie Ecologie, co-habilité Montpellier SupAgro et Université de Montpellier 2.

Je suis responsable de 3 Unités d'Enseignements et co-responsable de 4 unités d'enseignements :

M2 Ecosystèmes
- UE enjeux et controverses (4 semaines, 40 étudiants en moyenne), 5ECTS
- UE outils et méthodes du monde de la recherche (4 semaines, 20 étudiants en moyenne), 5ECTS
- UE cycles biogéochimiques (2 semaines, 5 étudiants en moyenne), 2,5 ECTS

M2 Ingénieur
- Géochimie et transferts des polluants (1 semaine, 10 étudiants en moyenne)
- Fonctionnement écologique des sols (2 semaines, 15 étudiants en moyenne), 3ECTS

M1 Ingénieur
- Ecologie de la vigne (5 semaines, 32 étudiants en moyenne), 2,5 ECTS
- Géochimie et écologie des sols (4 semaines, 15 étudiants en moyenne), 6ECTS

Mon enseignement présente la contribution de la géochimie au fonctionnement des écosystèmes. Je développe des enseignements relatifs aux cycles biogéochimiques, à la contribution des sols aux services écosystémiques et aux relations entre activité biologique et composition minérale et organique des sols. La répartition moyenne de mes enseignements[1] est la suivante :

- Licence (L3 ingénieur Montpellier SupAgro) : 89h éqTD
- Master (M1, M2 ingénieur et master recherche) : 127h éqTD
- Formation courte non rémunérée : 5h éqTD

Liste des étudiants ingénieurs encadrés entre 2010 et 2014 (5 étudiants)
- Ingénieur en césure (tuteur école, 2 étudiants)
- Ingénieur stage de M1 (tuteur école, 3 étudiants)

Participation à des jurys de soutenance diplômant entre 2010 et 2014

- Jury permanent de fin de stage de M1 et M2 recherche ECOSYSTEMES (environ 16 étudiants par an)
- Jury de stage de fin d'étude de licence professionnelle L3 Viticulture raisonnée (2 étudiants)
- Jury du M2 spécialité ingénieur (4 étudiants)
- Jury de master 3 A (1 étudiant)

ANIMATION DE LA RECHERCHE

o *Organisation de workshop, congrès*

Membre du comité local d'organisation de « 5th Phosphorus in Soils and Plants - PSP5 », 26 - 29 Août 2014, Montpellier, France
Membre du comité local d'organisation de « Sustainable Phosphorus Summit », 01 - 03 Septembre 2014, Montpellier, France
Membre du comité local d'organisation de « Rhizosphere 2 », 26 - 31 Août 2007, Montpellier, France

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[1] Calcul effectué hors années avec congé maternité

- *Participation à des groupes de travail*

2011. Séminaire de réflexion sur l'intensification écologique des agro écosystèmes, 29 Novembre – 01 Décembre, Dourdan la Forêt, France.

ACTIVITES COLLECTIVES

Lors du quadriennal 2011 – 2014, j'étais co-responsable de l'axe enseignement de l'UMR Eco&Sols. Je suis également membre nommée du conseil d'UMR.

Je suis membre élue du conseil des enseignants depuis 2012, et depuis 2013, je suis la représentante de Montpellier SupAgro, chargée par la directrice scientifique de Montpellier SupAgro, auprès du master Biologie Ecologie.

Je suis également la personne référente auprès de la Direction des Relations Internationales de Montpellier SupAgro pour des étudiants étrangers inscrits à SupAgro souhaitant avoir des renseignements sur l'UMR Eco&Sols. En 2013, je suis intervenue dans le groupe de réflexion « UMRisation » de Montpellier SupAgro sur demande du directeur scientifique, Bruno Blondin.

EXPERTISE

Depuis 2012, je suis membre élue du conseil scientifique de Montpellier SupAgro.

Participation en 2012 au lancement du Groupement d'Intérêt Scientifique « Vigne », thématique « gestion du capital sol en vigne ».

Liste des travaux et 5 Publications marquantes

LISTE DES TRAVAUX AVEC IDENTIFICATION DES ENCADREMENTS

Source IF 5 ans : 2013 JCR Science Edition Thomas Reuters

Articles scientifiques dans des journaux internationaux avec comité de lecture (total 16)(sont mis en couleur les noms des doctorants que j'ai co-encadrés)

[P 1] Trouillefou C.M., **Le Cadre E.**, Cacciaguerra T., Cunin F., Plassard C., Belamie E. 2014. Protected activity of a phytase immobilized in mesoporous silica benefits to plant phosphorus nutrition, article en cours de publication, Journal of Soil Gel and Technology (IF $_{5\ ans}$: 1,763)

[P 2] Salome C, Coll P, Lardo E, Villenave C, Blanchart E, Hinsinger P, Marsden C, **Le Cadre E**. 2014. Relevance of use-invariant soil properties to assess soil quality of vulnerable ecosystems : the case of Mediterranean vineyards. **Ecological Indicators.** 43, 83-93. (IF $_{5\ ans}$: 3,384)

[P 3] Devau N., Hinsinger P., **Le Cadre E.**, Colomb F. Gerard F. 2013. Reply to the Comment by D. G. Lumsdon on "Fertilization and pH effects on processes and mechanisms controlling dissolved inorganic phosphorus in soils" by N. Devau, P. Hinsinger, E. Le Cadre, B. Colomb and F. Gerard. **Geochimica et Cosmochimica Acta**. 124, 410-417 (IF $_{5\ ans}$: 4,798)

[P 4] Lardo E, Coll P, **Le Cadre E**, Palese AM, Villenave C, Xiloyannis C, Celano G. 2012. Electromagnetic induction (EMI) measurements as a proxy of earthworm presence in Southern French vineyards. **Applied Soil Ecology**. 61, 76-84 (IF $_{5\ ans}$: 2,952)

[P 5] Coll P, **Le Cadre E.**, Villenave C. 2012. How are nematode communities affected during conversion from conventional to organic farming in southern French vineyards. **Nematology.** 14, 665-676 (IF $_{5\ ans}$: 1,303)

[P 6] Hinsinger P, Brauman A, Devau N, Gérard F, JourdanC, Laclau JP, **Le Cadre E**, Jaillard B, Plassard C. 2011. Acquisition of phosphorus and poorly mobile nutrients by roots. Where do our plant nutrition models fail ? **Plant Soil**. 348, 29-61. (IF $_{5\ ans}$: 3,713)

[P 7] Devau N, Hinsinger P, **Le Cadre E**, Gérard F. 2011. Root induced process controlling phosphate availability with contrasted P-fertilized treatments. **Plant Soil.** 348, 203-218. (IF $_{5\ ans}$: 3,713)

[P 8] Coll P, **Le Cadre E**, Blanchart E, Hinsinger P, Villenave C. 2011. Organic viticulture and soil quality : a long term study in Southern France. **Applied Soil Ecology**. 50, 37-44. (IF $_{5\ ans}$: **2.952**)

[P 9] Devau N, Hinsinger P, **Le Cadre E.**, Colomb B, Gérard F. 2011. Fertilization and pH effects on processes and mechanisms controlling dissolved inorganic phosphorus in soils. **Geochimica et Cosmochimica Acta**. 75, 2980-2996. (IF $_{5\ ans}$: 4,798)

[P 10] Devau N, **Le Cadre** E, Hinsinger P, Gérard F 2010. A mechanistic model for understanding root-induced chemical changes controlling phosphorus availability. **Annals of Botany** 105, 1183-1197. (IF $_{5\ ans}$: 4,001)

[P 11] Devau N, **Le Cadre E**, Hinsinger P, Jaillard B, Gérard F. 2010. Soil pH controls the environmental availability of phosphorus: Experimental and mechanistic modelling approaches (vol 24, pg 2163, 2009). **Applied Geochemistry 25, 1094-1095.** (IF $_{5\ ans}$: 2,560)

[P 12] Devau N, **Le Cadre E,** Hinsinger P, Jaillard B and Gérard F. 2009. Soil pH controls the environmental availability of phosphorus: Experimental and mechanistic modelling approaches. **Applied Geochemistry** 24, 2163-2174. (IF $_{5\ ans}$: 2,560)

[P 13] **Le Cadre E,** Gérard F, Genermont S, Morvan T, Recous S. 2009. Which formalism to model the pH and temperature dependence of the microbiological processes in soils ? Emphasis on nitrification. **Environmental Modeling and Assessment**. 14, 539-544. (IF $_{5\ ans}$: 1,156)

[P 14] Li H, Shen J, Zhang F, Clairotte M, Drevon J J, **Le Cadre E**, Hinsinger P 2008. Dynamics of phosphorus fractions in the rhizosphere of common bean (Phaseolus vulgaris L.) and durum wheat (Triticum turgidum durum L.) grown in monocropping and intercropping systems. **Plant and Soil** 312, 139-150. (IF $_{5\ ans}$: 3,713)

[P 15] **Le Cadre E.**, Génermont S., Azam F., Recous S. **2004**. The SAHGA model to calculate the Spatial Ammoniacal Heterogeneity after Granule Application. **Biology and Fertility of Soils.** 40: 178-180 (IF 3.074)

[P 16] **Le Cadre E.**, Génermont S., Decuq C., Recous S., Cellier P. **2004**. Sources of uncertaintinities in estimating low ammonia volatilization fluxes in laboratory systems. **Agronomy and Sustainable Development,** 25, 101-107. (IF 3.363)

Articles scientifiques dans des journaux nationaux avec comité de lecture (total 4)

[P 17] Coll P., Le Velly R., **Le Cadre E.,** Villenave C. 2011. La qualité des sols : associer perceptions et analyses des scientifiques et des viticulteurs. Etude et Gestion des sols. 19, 79-89.

[P 18] Coll P., Le Cadre E., Villenave C. Evaluer la qualité des sols pour adopter des pratiques viticoles durables. Progrès Agricole et Viticole. 129, 445-448.

[P 19] Coll P., Le Cadre E., Villenave C. Quels effets à long terme de la viticulture biologique sur le fonctionnement du sol ? Progrès Agricole et Viticole. 129, 449-452.

[P 20] Coll P, Arnal D., Blanchart E, Hinsinger P, Le Cadre E., Souche G, Villenave C. 2009. Qualité des sols viticoles: bénéfices de l'enherbement permanent sur les proprieties chimiques et les indicateurs biologiques des sols. Progrès Agricole et Viticole. 126, 527-531.

Communications et posters dans des congrès internationaux avec comité de lecture et actes (total 6)

[C. 1] Vidal A. Plassard C., Clairotte M., Deleporte P., Abbadie J., Aznar JL, Le Cadre E. 2014. Factors controlling phytases produced by the fungi Aspergillus niger and Debaryomyces castellii in rhizosphere soils. 5th International Symposium Phosphorus in Soils and Plants, Aug 25th-29th, Montpellier France

[C. 2] Mareschal L., Koutika LS, Le Cadre E., Bouillet JP, Epron E, Laclau JP. 2014. Effects of introducing leguminous species on phosphorus and nitrogen avalaibility in mixed stand forest. 5th International Symposium Phosphorus in Soils and Plants, Aug 25th-29th, Montpellier France

[C. 3] Coll P., Lardo E., Le Cadre E., Villenave C., 2011. Studying soil quality is essential to evaluate the sustainability of a vineyard. 17th international Symposium GiESCO – Proceeding of Symposium, Asti- Alba (CN), Italy,29 Aug- 2 Sept, pp: 91-93. ISSN 0369-8173.

[C. 4] Devau N., Le Cadre E., Hinsinger P., Gerard F. 2010. Surface complexation modelling of phosphorus availability and bioavailability for plants. Conference on Goldschmidt 2010 – Earth, Energy and the Environment, June 13th-18th, Knoxville, USA.

[C. 5] Devau N., Gerard F., Le Cadre E., Roger L., Jaillard B., Hinsinger P.2008. Using a mechanistic adsorption model to understand the influence of soil pHon the environmental availability of phosphorus: applications to a Chromic Cambisol. 18th annual V M Goldschmidt Conference, Vancouver, Canada.

[C. 6] Li H., Shen J., Zhang F., Clairotte M., Drevon JJ, Le Cadre E., Hinsinger P. 2008. Dynamics of phosphorus fractions in the rhizosphere of common bean (Phraseolus vulgaris L.) and durum wheat (Triticum turgidum durum L.) grown in monocropping and intercropping systems. 2nd International Conference on Rhizosphere, Aug., Montpellier, France.

Brevets et enveloppes Soleau (total 1)

[B. 1] Enveloppe soleau N° 491116171013

Communications et posters dans des congrès nationaux avec comité de lecture et actes (total 1)

[C. 7] Hinsinger P, Jaillard B, Le Cadre E and Plassard C 2009 Speciation and bioavailability of phosphorus in the rhizosphere. In Oceanis, Vol 33, No 1 and 2 - Phosphore Dans L'environnement: Bilan Des Connaissances Sur Les Impacts, Les Transferts Et La Gestion Environnementale. Eds. J M Dorioz, P Aurousseau and G Bourrie. pp 37-50.

Communications internationales avec comité de lecture sans actes (total 7)

[C. 8] Coll P. Le Cadre E., Blanchart E., Arnal D., Hinsinger P., Villenave C. 2010. Nematofauna, a sensitive bioindicator to characterize global soil quality during organic conversion of a vineyard, 30th International ESN Symposium, Sept 19th-23rd, Vienna, Austria. (communication orale)

[C. 9] Hinsinger P, Bravin M N, Devau N, Gerard F, Le Cadre E, Jaillard B 2008 Soil-Root-Microbe Interactions in the Rhizosphere - A Key to Understanding and Predicting Nutrient Bioavailability to Plants. Revista De La Ciencia Del Suelo Y Nutricion Vegetal 8. (communication orale)

[C. 10] Arshad, M.; Le Cadre E., Hinsinger P., Jaillard B. 2006. Interactions between soil pH buffer capacity and phosphorus bioavailability, 3nd International Symposium on Phosphorus Dynamics in the Soil-Plant Continuum. Uberlândia-Minas Gerais, Brazil , 14-19th May 2006 (poster). .

[C. 11] **Le Cadre E.**, Génermont S., Bedos C., Recous S. **2003.** Modelling ammonia volatilisation from synthetic fertilizers. 12th N workshop, 21-24 September, Exeter UK.(poster)

[C. 12] **Le Cadre E.**, Génermont S., Recous S. **2003**. Dissolution of granular fertilizers in soil and its effect on ammonia volatilisation. 12th N workshop, 21-24 September, Exeter UK. (poster)

[C. 13] **Le Cadre E.**, Lasserre-Joulin F.,Vong P.C., Guillemin S., Xardel S., Guckert A. **2002.** Distribution of 35S in field-grown oilseed rape vegetative compartments under different sulphur-fertilizer inputs. Congress of the European Society of Agronomie, 15-18 July, Cordoba, Spain. 845 p. (poster)

[C. 14] **Le Cadre E.**, Jeuffroy M.H., Génermont S., Flura D., Barbottin A., Chapoulie E. **2001.** Reduce ammonia volatilization to increase N use efficiency of a wheat crop. 11th N workshop, 10-13 September, Reims, France. (poster)

Communications et posters nationaux avec comité de lecture sans actes (total 1)

[C. 15] Le Cadre E., Génermont S., Azam F., Recous S. 2004. Microsites et hétérogénéité spatiale de l'azote ammoniacal et du pH après apport de fertilisants solides au champ. Journées Nationales d'Etude du Sol. 24-26 octobre, Bordeaux, France. (oral)

Communications et posters nationaux sans comité de lecture sans actes (total 6)

[C. 16] Lardo E., Coll P., Le Cadre E., Palese A.M., Xiloyannis C., Villenave C., Blanchart E., Ferrazzano G., Celano G., 2012. L'uso della tecnica EMI in pescheto per lo studio della variabilità spaziale degli indicatori biologici di qualità del suolo. VII PESCHMED, Lamezia T. (CS), Italy 26-27 Mag., Acta Italus Hortus, ISSN: 1127-3496.

[C. 17] Lardo E., Palese A.M., Coll P., Le Cadre E., Villenave C., Nuzzo V., Xiloyannis C.,Celano G. Relazioni tra Elettromagnetismo Indotto (EMI) e popolazioni di lombrichi in vigneti della Francia meridionale (accettato al IV COnvegno NAzionale di VIticoltura, Torino, Luglio - 2012). Acta Italus Hortus, ISSN: 1127-3496.

[C. 18] Lardo E., Coll P., Celano C., Le Cadre E., Villenave C., Xiloyannis C., 2011. Soil organic matter, aggregate stability and respiration: integrated measures for the evaluation of the soil sustainability in the vineyard. 17th international Symposium GiESCO - Proceeding of Symposium, Asti-Alba (CN), Italy,29 Aug- 2 Sept, pp: 155-158. ISSN 0369-8173.

[C. 19] Lardo E., Palese A.M., Coll P., Le Cadre E., Villenave C., Xiloyannis C., Celano G. Soil quality evaluation of fruit crop systems in semi-arid climatic conditions (accettato al 2nd Symposium on horticulture in a changing world, SHE2012, Angers, France). ISHS Acta Horticulturae, ISSN 0567-7572.

[C. 20] Le Cadre E. 2004. Modélisation de la volatilisation d'ammoniac en interaction avec les processus biologiques et chimiques du sol. Congrès de l'Association Française d'Etude du sol (AFES). 24.06.2004, Paris, France (oral)

[C. 21] Génermont S., Jeuffroy M.H., Le Cadre E., Hénault C., Laville P., Recous S. 2004. Les émissions gazeuses après fertilisation minérale : une perte d'azote pour la culture. Colloque national de la gestion de l'azote, avancées scientifiques. Académie Française d'Agriculture. 5.février, Paris, France. (oral)

CHOIX DE 5 PUBLICATIONS MARQUANTES

Le choix des publications marquantes de ma carrière est le suivant. Elles sont marquantes pour différentes raisons, que j'explique ci après :

Publication n°1 : Trouillefou C.M., Le Cadre E., Cacciaguerra T., Cunin F., Plassard C., Belamie E. 2014. Protected activity of a phytase immobilized in mesoporous silica benefits to plant phosphorus nutrition, acceptée sous réserve de modifications à Journal of Soil Gel Science and Technology (IF $_{5ans}$: 1.763).

L'histoire d'un pari risqué, celui d'une collaboration improbable entre la chimie des matériaux, la géochimie et la physiologie végétale. J'adresse ici ma gratitude à Christophe Trouillefou qui n'a pas tremblé devant le risque que nous lui faisions prendre en lui proposant ce sujet de thèse. A l'issu de ses travaux, de nouvelles perspectives s'ouvrent auxquelles j'envisage un potentiel intéressant.

Publication n°2 : Salome C, Coll P, Lardo E, Villenave C, Blanchart E, Hinsinger P, Marsden C, Le Cadre E. 2014. Relevance of use-invariant soil properties to assess soil quality of vulnerable ecosystems : the case of Mediterranean vineyards. Ecological indicators. 43, 83-93 (IF $_{5ans}$: 3.384).

Cette publication est issue des données de terrain collectées lors de la thèse de Patrice Coll. Clémence Salomé a repris en post doctorat la base de données passée au cribles des statistiques. C'est aussi la première publication dans une revue d'écologie qui symbolise le virage de mon projet de recherche. Enfin, la stratégie de collecte de données de terrain lors des thèses de Patrice Coll et Egidio Lardo s'est finalement justifiée par cette publication et associe donc ces deux doctorants.

Publication n°3 : Coll P, Le Cadre E, Blanchart E, Hinsinger P, Villenave C. 2011. Organic viticulture and soil quality : a long term study in Southern France. Applied Soil Ecology. 50, 37-44 (IF : 2,952).

Cette publication, associée à une deuxième a amorcé ma réflexion sur l'utilisation du concept de qualité des sols pour l'évaluation de la disponibilité des nutriments. Je poursuis cette réflexion dans le cadre de mon projet de recherche.

Publication n°4 : Devau N, Le Cadre E, Hinsinger P, Jaillard B and Gérard F. 2009 Soil pH controls the environmental availability of phosphorus: Experimental and mechanistic modelling approaches. Applied Geochemistry 24, 2163-2174 (IF : 2,560).

Une première publication qui sera suivie de plusieurs autres. Lors de cette thèse, tout n'a pas été facile car ces travaux m'ont demandé un virage thématique vers la géochimie mécaniste. Au final, malgré les difficultés rencontrées, la réflexion entamée lors de cette thèse m'a permis de développer mon projet de recherche et de réfléchir à de nouveaux enseignements.

Publication n°5 : Le Cadre E, Gérard F, Genermont S, Morvan T, Recous S. 2009. Which formalism to model the pH and temperature dependence of the microbiological processes in soils ? Emphasis on nitrification. Environmental Modeling and Assessment. 14, 539-544. (IF $_{5 ans}$: 1,156)

Une publication qui a permis de faire le point sur les formalismes existants pour tenir compte des effets du pH et la température sur les réactions biologiques des sols. Dans cette publication, nous avons utilisé un formalisme de bactériologie médicale prédictive qui s'est avéré puissant pour décrire la nitrification dans les sols et son rétro contrôle par les modifications de pH induits par les engrais de synthèse après application.

Bilan des activités de recherche 2005 - présent

Naturellement, la productivité des écosystèmes terrestres est limitée par les principaux éléments constitutif des plantes soit l'azote (N) , le phosphore (P) et le potassium (K) (Carpenter 2005; Tilman et al. 2002; Vitousek et al. 2010). Ainsi, (Loreau et al. 2001) estiment que la disponibilité des nutriments structure en partie les communautés végétales et (Finzi et al. 2011) attribue la modulation des réponses des plantes à des perturbations comme le changement climatique à leur capacité à acquérir des nutriments. Les réseaux trophiques aériens influencent et dépendent des réseaux trophiques du sol (Wardle et al. 2004). En effet, les plantes par leurs racines, *via* les exsudats racinaires, ou les parties aériennes revenant au sol, sont une entrée de carbone pour les organismes du sol et constituent alors la base des réseaux trophiques du sol. Les réseaux trophiques du sol avec leurs multiples interactions biotiques et abiotiques contribuent donc à la régulation du cycle des nutriments et à la croissance des plantes (Sylvain and Wall 2011). La compréhension des liens entre le cycle de la matière organique et le fonctionnement des écosystèmes est un enjeu majeur de l'écologie fonctionnelle (Bornette and Lagadeuc 2012). Cet enjeu de l'écologie fonctionnelle est repris désormais par l'agronomie afin de mieux comprendre le fonctionnement des agro-écosystèmes dans le but de les gérer durablement (Doré et al. 2011). En effet, à la fin des années 60, l'intensification des agro-écosystèmes a permis l'augmentation de la production céréalière dans le monde très largement grâce à l'utilisation des engrais. L'objectif de la fertilisation est d'augmenter la disponibilité des nutriments pour lever la limitation naturelle de la productivité des écosystèmes. L'application massive d'engrais a, en retour, contribué à l'augmentation de l'effet de serre (Sutton et al. 2011), la perte de biodiversité (Payne et al. 2013; Sutton et al. 2011)) ou la contamination des eaux (Withers et al. 2014). Au delà des considérations agronomiques et environnementales de l'utilisation des engrais, d'autres problématiques de raréfaction de la matière première des engrais émergent actuellement. En effet, parmi les 3 nutriments majeurs, seuls le phosphore et le potassium sont issus de l'altération chimique de matériaux de nature géologique et donc de nature finie (Cordell et al. 2009; Manning 2010; Roemheld and Kirkby 2010). Le constat actuel d'impasse économique et environnementale des modes actuels de production agricole impose un nouveau paradigme : la nécessité d'une intensification écologique des agro-écosystèmes pour limiter le recours aux intrants, et lorsqu'on parle de nutrition des plantes, de fertilisants.

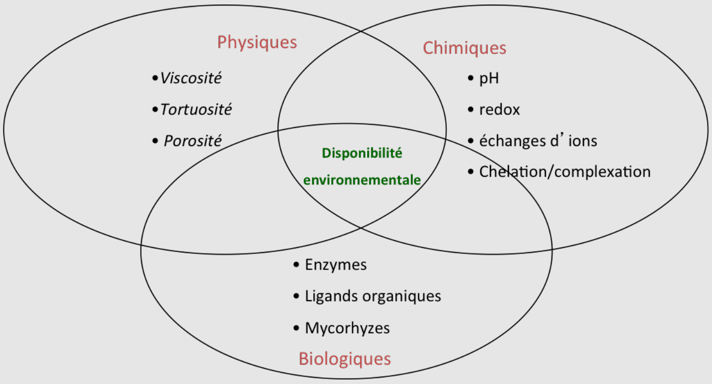
La disponibilité des nutriments est donc une question de recherche importante aux multiples enjeux écologiques et agro-environnementaux.

L'acquisition d'éléments par une plante fait appel aux concepts de disponibilité et biodisponibilité Harmsen et al. 2005 ; Harmsen 2007 ; ISO 2006). La disponibilité représente la concentration en élément présente dans le sol susceptible d'être acquise par une plante. La disponibilité est donc une fraction de la concentration totale du sol. La biodisponibilité est le flux d'élément vers la surface du système racinaire d'une plante cible cultivée sur un sol donné. La biodisponibilité a deux composantes : l'une physico chimique ou disponibilité environnementale et l'autre biologique, qui est l'aptitude d'une plante à acquérir l'élément présent dans le sol. La disponibilité environnementale regroupe un ensemble d'espèces chimiques (spéciation), minérales ou organiques susceptibles d'alimenter la concentration de l'élément dans la solution du sol. Les différentes espèces chimiques se distribuent dans les différentes phases du système (partition). Il est désormais admis que les plantes sont capables d'influencer leur milieu et notamment au travers de processus biogéochimiques contrôlant la disponibilité des éléments (Hinsinger 2001; Hinsinger et al. 2009; Hinsinger et al. 2011b; Hinsinger et al. 2005). Ces processus sont fondamentaux car seules certaines formes sont absorbées par les plantes comme les ions orthosphosphates pour le phosphore (Hinsinger 2001), ou l'ion K+ (Britto and Kronzucker 2008), alors que pour l'azote une plus grande variété d'espèces chimiques sont prélevées (Jones et al. 2005).

La compréhension des facteurs de régulation des composantes physico chimiques et biologiques de la biodisponibilité des éléments est une question de recherche majeure aux multiples enjeux agronomiques, écologiques et environnementaux.

Dans le cas des agroécosystèmes, la résidusphère et la rhizosphère sont des lieux privilégiés de transformation de la matière organique et de flux d'éléments (Bundt et al. 2001). Certains auteurs (Hinsinger et al. 2005) ont montré qu'un grand nombre de processus se déroulant dans la rhizosphère, c'est à dire le volume de sol adjacent aux racines et influencé par l'activité de ces dernières, modifie la disponibilité environnementale des éléments (Encadré 1).

Encadré 1 : résumé des modifications induites par les racines des plantes pour modifier la disponibilité environnementale des éléments dans leur rhizosphère



Comment estimer la disponibilité des nutriments ?

L'estimation de la disponibilité des nutriments repose le plus souvent sur des extractions chimiques, dont la pertinence est critiquable (Koopmans et al. 2004). Par exemple, les extractions chimiques utilisées pour estimer la disponibilité en phosphore des sols sont incapables de prendre en compte les modifications du milieu par les racines des plantes et les micro-organismes associées. La complexité de la géochimie des éléments nécessite de faire appel à de nouvelles méthodes et à la modélisation des réactions chimiques influencées par les êtres vivants. La modélisation permet d'étudier les interactions entre les processus ou les facteurs, contrairement à la démarche expérimentale qui ne permet que l'étude d'un nombre restreint de facteurs ou processus à la fois. Toutefois, il est utopique de penser que l'ensemble de ces réactions (voir Encadré 1) puisse être modélisé. Il est nécessaire d'établir une hiérarchie des facteurs afin de se concentrer sur les principaux facteurs pour examiner les interactions entre processus chimiques et biologiques régulant la disponibilité des nutriments tout en continuant les développements méthodologiques expérimentaux.

Afin de contribuer à comprendre comment les interactions entre les composantes abiotiques du sol et l'activité biologique des sols influencent la disponibilité des macro-nutriments N, P et K, j'ai défini 3 axes de recherches. L'ambition est d'identifier les stratégies de gestion des intrants limitant l'empreinte écologique de l'Homme sur les écosystèmes cultivés terrestres.

* * *

L'intensification écologique des agro-écosystèmes repose sur l'utilisation des fonctions naturelles des sols (Doré et al. 2011). Parmi ces fonctions, la capacité des plantes à modifier la géochimie des éléments afin d'augmenter la disponibilité environnementale est particulièrement pertinente. Ainsi, la diversité des plantes, en utilisant les interactions positives entre espèces (facilitation et complémentarité de niche) peut permettre d'augmenter la disponibilité du phosphore (Hinsinger et al. 2011a). D'autres auteurs (Jackson et al. 2008) ont également mis en avant la complexité des processus dans la rhizosphère pouvant affecter les formes d'azote assimilable par les plantes. La rhizosphère est donc un lieu privilégié d'étude pour identifier les meilleures interactions biotiques et abiotiques augmentant la disponibilité des nutriments. Dans ce contexte, j'ai formulé mon premier axe de recherche comme suit :

Axe 1. Peut on identifier et prédire l'effet de facteurs de régulation de la disponibilité environnementale des éléments : application au phosphore ?

Liste des projets de l'axe 1 : Projet 1, Projet 6, Projet 9
Publications associées de l'axe 1 :[P 13], [P 15], [P 16], [P 7], [P 9], [P 10], [P 12], [P 14]
Encadrement de la recherche de l'axe 1 : Devau N (M2, thèse), Trouillefou C (M2, thèse), Arshad M (M2), Veteló C (M2), Vidal A (M2), Julbe J (CDD)

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L'agriculture, par ses pratiques de gestion du sol peut influencer la diversité et l'abondance des organismes du sol (Barrios 2007; Wagg et al. 2014), mais également les états physiques et chimiques du sol interférant avec les réactions biologiques. Ainsi, quelques auteurs comme (Doré et al. 2011) insiste sur la pondération de l'importance des processus rhizosphériques lorsqu'il sont intégrés aux pratiques agricoles. Il convient donc d'examiner le poids relatifs des différentes interactions biotiques et abiotiques régulant la disponibilité environnementale à d'autres échelles que celle du segment de racine. Il faut une évaluation à l'échelle de la parcelle en association avec les techniques de gestion du sol pour proposer de nouvelles stratégies de gestion des intrants. C'est le deuxième axe de mes recherches :

Axe 2. Etude conjointe de la disponibilité environnementale et du fonctionnement du sol : application du concept de qualité des sols.

Liste des projets de l'axe 2 : Projet 3, Projet 5, Projet 7
Publications associées de l'axe 2 : [P 2], [P 4], [P 5], [P 8], + 1 article en préparation
Encadrement de la recherche de l'axe 2 : Salomé Clémence (post doctorat), Coll Patrice (doctorant), Lardo Egidio (PhD international 1,5 an en France), Gorbyk M. (M2)

* * *

Le développement de techniques d'agriculture de précision ou les itinéraires techniques basés sur les interactions biologiques et relevant de l'intensification écologique est nécessaire. Le dénominateur commun de ces stratégies techniquement bien différentes est de limiter, voire d'éliminer, les apports de formes minérales solubles et de mieux valoriser les ressources du sol ou les déchets (Makowski et al. 2014). L'industrie des engrais a donc un défi important à relever pour s'adapter aux futurs changements de pratiques des agriculteurs, ou d'anticiper les réglementations de protection de l'environnement comme le protocole de Göteborg. La recherche-développement de formulation d'engrais azotés a permis le développement de produits à libération contrôlée (Slow Release ou Control Release Fertilizers) parfois comprenant des inhibiteurs de la nitrification pour limiter le lessivage des ions nitrate ou les émissions de gaz azotés (Menendez et al. 2012; Menendez et al. 2006; Scheer et al. 2014; Weiske et al. 2001). Face à la pénurie programmée d'engrais solubles à base de P et K, il devient urgent d'explorer les alternatives de fabrication d'engrais qui pourraient accompagner une agriculture productive utilisant les concepts de l'écologie. Les éléments P et K ne bénéficient pas encore d'une recherche-développement de qualité suffisante en ce domaine dans la recherche publique. Certaines alternatives sont testées comme l'utilisation des roches broyées mais les résultats sont parfois peu concluants (Bolland and Gilkes 1990; Manning 2010). Récemment, certains auteurs (Withers et al. 2014) évoquent l'urgence de développer une recherche-développement de nouvelles formulations d'engrais. A chaque avancée des connaissances du fonctionnement des écosystèmes correspond une innovation technologique que l'on peut traduire en recherche finalisée voire appliquée. Ces opportunités doivent être transférables vers les acteurs économiques. Mon dernier axe de recherche, en partenariat avec divers acteurs du secteur économique a pour ambition de développer les résultats des axes 1&2 dans des innovations technologiques ou de vulgarisation. Les valorisations peuvent être des enveloppe Soleau, des brevets, voire non divulgués à l'INPI[2] car faisant partie du secret industriel.

Axe 3. Recherche d'applicabilité des travaux

Liste des projets de l'axe 3: Projet 2, Projet 8, Projet 4
Publications associées de l'axe 3 : [P 1], [P 15]   [B. 1], deux brevets en cours de rédaction
Encadrement de la recherche de l'axe 3 : Trouillefou C. (doctorant), Coll P. (doctorant), Lardo E. (doctorant), Grondin Jennifer (CDD ingénieur d'étude), Julbe Justine (CDD ingénieur d'études), Benoit Simon (CDD ingénieur d'étude),

* * *

D'un point de vue disciplinaire, mes travaux se situent dans le champ de l'écologie fonctionnelle à l'interface avec la géochimie. Ma stratégie de recherche consiste à combiner approche expérimentale de terrain et de laboratoire avec de la modélisation (mécaniste et empirique). Pour mener à bien ma stratégie de recherche, j'utilise des modèles et méthodes existantes mais je propose également de nouvelles approches méthodologiques et formalismes de modélisation.

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[2] Institut National de la Propriété Industrielle

Axe 1. Peut on identifier et prédire l’effet de facteurs de régulation de la disponibilité environnementale des éléments : application au phosphore ?

Introduction

La disponibilité des espèces chimiques de l'azote, phosphore et potassium est sous le contrôle de différentes réactions chimiques et biologiques (Hinsinger et al. 2011c; Husson 2013; Manning 2010). En prenant l'exemple du pH comme facteur de régulation, l'expérimentation peut contribuer à évaluer les effets du pH sur la disponibilité des éléments dans certains hot spots comme la rhizosphère (Hinsinger et al. 2003) grâce à des techniques adaptées (Blossfeld et al. 2013). Toutefois, la généricité de ces résultats expérimentaux doit être testée à l'aide de modèles de prédiction de la disponibilité en nutriments intégrant les effets directs sur l'activité biologique et les effets en retour de celle ci sur le pH.

Démarche

Une approche unique ne pourrait être employée pour évaluer l'effet des facteurs de régulation de la disponibilité en nutriments. J'ai utilisé à la fois la modélisation mécaniste à base de données (i) issues de la littérature, (ii) acquises expérimentalement ou (iii) adapté des formalismes de disciplines comme la bactériologie prédictive. Ma stratégie générale est de choisir les formalismes les plus adaptés pour l'intégration dans des modèles mécanistes incluant les réactions chimiques voire les transferts. Cette stratégie, qu'on peut qualifier de semi mécaniste permet une évaluation globale des processus impliqués dans la disponibilité des éléments.

Principaux résultats

En thèse, puis suite à mon recrutement à Montpellier Supagro, je me suis intéressée à la prédiction disponibilité des formes minérales de l'azote (ammonium et nitrate) après application d'engrais de synthèse pour évaluer la contribution de ces engrais aux émissions d'ammoniac d'origine agricole. La disponibilité en ammonium et nitrate dépend des processus d'organisation, minéralisation (cycle interne de l'azote) et nitrification. Ces réactions dépendent de l'activité biologique des sols et des propriétés du sol comme la texture, l'humidité et le pH (Jackson et al. 2008). Ces réactions ne sont pas réalisées par les mêmes organismes du sol et par conséquent les facteurs de sensibilité à la température ou au pH sont différents. Dans le cas de la prédiction des émissions d'ammoniac après fertilisation, il faut conceptualiser les interactions biotiques et abiotiques impliquées dans la volatilisation de l'ammoniac. Par exemple, (Praveen-Kumar and Aggarwal 1998) ont démontré que la volatilisation d'ammoniac et la nitrification sont interdépendantes. En effet, la volatilisation se termine quand débute la nitrification. La nitrification est inhibée par de trop fortes concentrations en ammonium et par conséquent la volatilisation d'ammoniac lève ainsi l'inhibition de la nitrification. Je devais tenir compte également des cinétiques très rapides des processus induisant des variations de pH. Pour cela, j'ai modélisé les réactions tampons du pH à court terme (non publié) et repris les travaux de (Rosso et al. 1995; Rosso et al. 1993) en bactériologie prédictive médicale afin de tenir compte explicitement de la

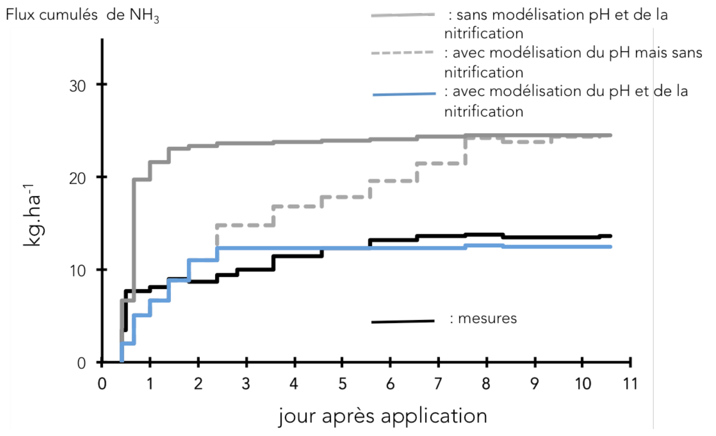


Figure 1 : simulation du modèle VOLT'AIR après implémentation des différentes réactions influençant le cycle de l'azote après application de solution d'ammonium nitrate au champ.

taille de la population de bactéries nitrifiantes et du pH [P 13]. J'ai également pris en compte explicitement la forme d'application (solide ou liquide) des engrais [P 15] et validé les résultats au moyen d'une mise au point expérimentale [P 16]. Pour l'hydrolyse de l'urée, l'analyse de la littérature sur l'activité de cette exo-enzyme a permis une formalisation. Le principal résultat est présenté dans la Figure 1, où je montre que la prise en compte explicite des interactions entre processus chimiques et biologiques améliore nettement la prédiction des flux d'ammoniac après application d'une solution d'ammonium nitrate au champ. Nous avons également pu démontrer que la modélisation du pH était critique. Les différences observées entre le modèle et les mesures ont été attribuées à une mauvaise prédiction de la teneur en eau des premiers centimètres de sol à l'aide du modèle de bilan d'énergie. En effet, la modélisation des effets de la teneur en eau sur les réactions biologiques comme la nitrification et l'activité uréasique sont encore imparfaites aux échelles de temps courtes.

La modélisation des variations de pH permet une estimation plus précise du pool d'azote ammoniacal mais qu'en est il pour les autres éléments ? Application au phosphore

A mon recrutement, je me suis intéressée à une autre élément, le phosphore, élément modèle de l'UMR d'accueil. Les ions phosphates, seule forme absorbable par les plantes et l'ammonium ont en commun leur forte réactivité avec la phase solide du sol. En thèse, j'avais tenu compte des réactions à l'interface solide liquide uniquement par une isotherme d'adsorption. Or les isothermes d'adsorption ont une valeur prédictive faible et ne peuvent tenir compte des effets du pH sur la modification de charge de surface des colloïdes impliqués dans les phénomènes de sorption (Limousin et al. 2007). Le pH intervient en effet dans la plupart des réactions chimiques en phase aqueuse et contrôle la charge de surface des colloïdes du sol impliqués dans les réactions chimiques de sorption (Sigg et al. 2006). Parmi les différents facteurs influençant la disponibilité, le pH est commun à la régulation des 3 grands cycles biogéochimiques de l'azote (N), phosphore (P) et potassium (K) par son contrôle des réactions physico chimiques de sorption, précipitation/dissolution et d'hydrolyse. Concernant les réactions biologiques, le pH affecte la biogéographie et l'activité des communautés microbiennes (Baggs et al. ; Dequiedt et al. 2011; Regan et al. 2014) ainsi que l'activité des exo-enzymes libérées par les communautés microbiennes ou fongiques (George et al. 2005; Giaveno et al. 2010; Klose and Tabatabaï 1999; Klose 2000; Makboul and Ottow 1979; Rao et al. 2000; Rao 1983). Afin d'estimer le poids du pH par rapport à d'autres facteurs de régulation de la disponibilité en éléments, j'ai structurée mon premier axe en 3 objectifs :

Objectif 1.1. Estimation expérimentale du poids des variations de pH par rapport à d'autres activités racinaires sur la disponibilité du P. (M2 Arshad M.)

Objectif 1.2. Identification des réactions chimiques contrôlées par le pH définissant la disponibilité environnementale du P mesuré en objectif 1.1 à l'aide d'un modèle de complexation de surface à l'équilibre (Visual Mineteq), puis hiérarchisation du poids du pH. (M2 Devau N.)

Objectif 1.3. Estimation du poids du pH par rapports à d'autres processus rhizosphériques sur différents sols et identification des phases solides du sol contrôlant la disponibilité du phosphore (thèse Devau N.)

Notre modèle biologique est le blé dur (*Triticum turgidum durum*), plante capable de modifier le pH de sa rhizosphère de 1 à 3 unités pH, et les sols sont un Chromic Cambisol prélevé à Cazevielle dans le département de l'Hérault (objectif 1.1 & 1.2) et un Luvisol (objectif 1.3) prélevé à Toulouse-Auzeville (Haute Garonne). Ces sols sont de minéralogie contrastées et ont des teneurs en matière organique et P total différentes. Sur la base des travaux de (Alamgir et al. 2012; Kuczak et al. 2006; Malik et al. 2012), nous avons d'abord utilisé une approche expérimentale utilisant les extractions séquentielles de P afin de déterminer l'évolution des pools de P susceptibles d'intervenir dans la nutrition des plantes et d'être influencés par l'activité racinaire. Un dispositif de culture original a été utilisé afin de récupérer un maximum de sol rhizosphérique comme dans l'article de (Li et al. 2008) [P 14].

Les résultats obtenus dans les objectifs 1.1 et 1.2 ont permis de mettre en évidence que les formes Pi disponibles extraites par des résines anioniques (Guo et al. 2000) sont modifiées par le pH seul alors que les formes estimées moyennement ou peu disponibles ne le sont pas comme le Pi residue (Figure 2). En présence de plants de blé, Arshad M. et Devau N. ont mesuré une modification du pH de leur rhizosphère sans que cette modification de pH n'induise de modification du pool des formes de P rapidement mobilisable (Pi résine). Par contre, les plantules modifient les pools de P très peu disponibles (P residue) ou organiques (extrait NaOH), données non présentées. L'hypothèse formulée est celle d'une mobilisation du P de ces formes vers les formes extractibles avec des résines. Le pH est donc une variable de régulation de la disponibilité en phosphore du sol de formes rapidement disponibles, mais les plantes peuvent avoir accès à d'autres formes de P.

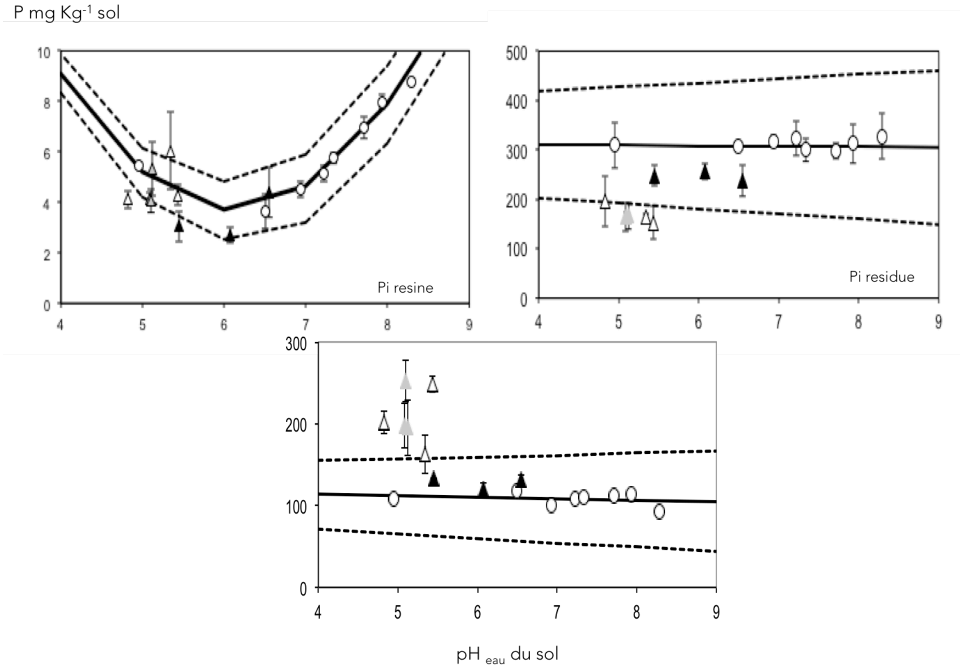


Figure 2 : comparaison de deux formes de phosphore extraites du Chromic Cambisol après modification du pH sans plante (courbe pleine) et après modifications du pH par la rhizosphère par deux variétés de blé (symboles) cultivées avec de l'azote ammoniacal comme source d'azote principale. Les formes de phosphore sont obtenues après extraction séquentielle (adapté du protocole de Hedley décrit dans (Li et al. 2008) [P 14]). Données non publiées issus des travaux de Arshad M. et Devau N.

Afin de caractériser la nature des processus géochimiques contrôlant la disponibilité en phosphore inorganique, nous avons combiné cette approche expérimentale avec une approche de modélisation mécaniste. L'approche de modélisation choisie est originale car elle repose sur la modélisation additive des propriétés et réactions de chacun des constituants du sol (argiles, oxydes de fer et d'aluminium, matière organique) vis à vis des ions orthophosphates. Notre choix s'est porté sur les modèles 1-pK Triple Plane Mode, échange d'ion et le formalisme de NICA-DONNAN. Le modèle choisi est un modèle dit à l'équilibre, Visual Minteq (Gustafsson, KTH Suède). Cette approche a été choisie pour caractériser la nature des processus géochimiques contrôlant la disponibilité en phosphore inorganique et évaluer le rôle du pH dans ces derniers. Nous avons fait le choix de mesurer et simuler la disponibilité en P à partir d'extraction $CaCl_2$ (0,01M) plutôt que de l'eau car elles modifient peu les conditions chimiques, notamment le pH contrairement aux méthodes à base de résines échangeuses d'anions (Myers et al. 2005) lorsqu'elles sont conditionnés par des ions bicarbonates. Ce type d'extraction rendent bien compte de la quantité de phosphore disponible régulé par le processus d'adsorption - désorption.

De cette manière, nous avons pu démontrer que le processus d'adsorption-désorption contrôlait la disponibilité des ions orthophosphates en solution ([P 12], [P 9]) pour une large gamme de pH de sol (pH 4 à pH 10). Nous avons testé notre hypothèse avec le sol de Cazevielle ([P 12]), puis confirmée avec succès sur l'autre sol, le Luvisol ([P 9]). En effet, dans le cas de ce dernier, nous avons utilisé un essai de longue durée de fertilisation phosphatée (Colomb et al. 2007), permettant de tester le rôle de la quantité de phosphore inorganique total disponible. Nous avons également pu identifier la contribution des phases solide du sol (kaolinite et illite) à ce mécanisme d'adsorption en fonction du pH (Figure 3). Nous avons pu mettre en évidence l'effet inattendu des électrolytes (Ca^{2+} et Cl^{-}) en fonction du pH. Pour de fortes valeurs en P et calcium, le processus de précipitation de l'hydroxyapatite peut également contribuer à contrôler la disponibilité du P.

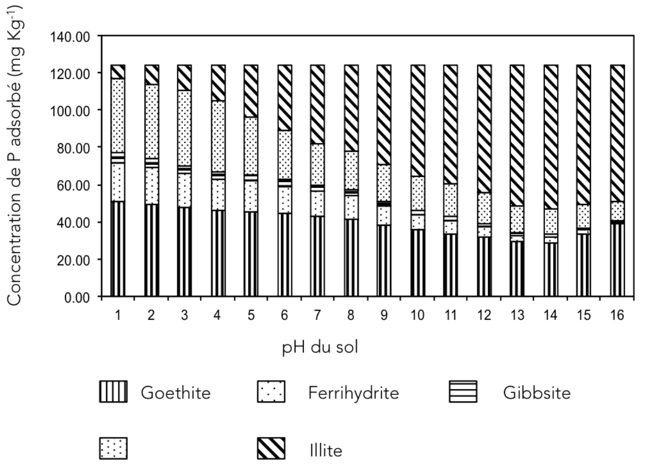


Figure 3 : concentrations de phosphore adsorbé en fonction du pH sur les différentes minéraux du sol (adapté de (Devau et al. 2009)).

Cette approche n'aurait pas été complète sans déterminer l'effet de la modification de pH dans la rhizosphère du blé dans chacun de ces deux sols. La culture de blé menée à l'aide des dispositifs de type « Rhizobox » a alors été utilisée comme dans le M2 de Arshad M. Les résultats relatifs à ces travaux sont décrits dans les publications [P 10] et [P 7].

En conclusion, nous avons démontré comment le pH en contrôlant l'adsorption des ions orthophosphates contribuait à réguler la disponibilité du phosphore disponible.

De la disponibilité à la biodisponibilité : un autre regard sur la contribution du pH

Un autre résultat marquant obtenu lors de cette deuxième étape, réalisée dans la thèse de N. Devau, concerne la mise en évidence d'un nouveau processus rhizosphérique, le prélèvement en Ca, susceptibles d'induire une augmentation de la disponibilité en P dans la rhizosphère du blé dur selon le pH et la concentration en Ca du milieu. Les travaux de N. Devau ont permis de mettre en évidence que le prélèvement de Ca augmentait la disponibilité de P en modifiant l'influence du processus d'adsorption-désorption du Ca sur celui du P. Les limites de notre approche reposent sur une calibration des surfaces spécifiques des minéraux correspondant à des structures relativement bien cristallisées. Or, ce n'est certainement pas le cas dans les sols. Malgré ces limites, les travaux de N. Devau ont soulevé plusieurs pistes de réflexion pour améliorer notre compréhension de la biodisponibilité du phosphore. Par exemple, l'effet de l'exsudation d'anions organiques n'a pas été testé alors que (Jones et al. 2003) et (Richardson et al. 2009) mettent en avant les effets de compétition entre anions organiques et ions orthophosphates. Cette approche a récemment été testée avec succès par (Duputel et al. 2013a; Duputel et al. 2013b) dans notre unité de recherche.

J'ai choisi à l'issue de la thèse de N. Devau, une approche complémentaire à celle de mes collègues en m'intéressant à l'hydrolyse enzymatique de formes de phosphore organique dans la rhizosphère des plantes. En effet, les enzymes sont essentielles pour le cycle des nutriments dans le sol et donc leur disponibilité pour les plantes et les organismes du sol en général. De plus, les activités des enzymes du sol, qu'elles soient intra- ou extra-cellulaires, sont de bons indicateurs des modifications de fonctionnement du sol car ils rendent compte de l'activité des organismes les synthétisant à savoir les bactéries, champignons et plantes (Naseby and James 2002). Les enzymes impliquées dans la dégradation d'une forme de phosphore organique, le phytate ou myo-inositol hexa-phosphate sont particulièrement intéressantes. Dans la rhizosphère des plantes, l'activité enzymatique est souvent plus élevée que dans le sol non influencé par l'activité des racines grâce à l'ajout de carbone exsudé par les plantes stimulant l'activité microbienne (Renella et al. 2007). L'activité des uréases et des phytases comportent des similarités parmi les enzymes intra- ou extra-cellulaires. Lorsqu'elles sont émises dans le milieu, leur activité peut être modifiée par les constituants du sol (Beri 1978; Bremner 1976; Gould et al. 1973; Grant et al. 1996; Klose and Tabatabaï 1999; Klose 2000; Makboul and Ottow 1979; McGarity and Myers 1967; McNaughton et al. 1997; Moyo et al. 1989; Myers and McGarity 1968; Nannipieri et al. 1978; Pal and Chhonkar 1981; Pettit et al. 1976; Reynolds and Wolf 1987; Zantua et al. 1977) et plus récemment pour les phosphatases et phytases (George et al. 2005; George et al. 2007b; Giaveno et al. 2010; Shi et al. 2009). Les plantes ont peu de capacités à hydrolyser le phytate fourni comme seule source de phosphore car elles ne sont pas capables de sécréter la phytase dans le milieu rhizosphérique. Toutefois, lorsqu'elles sont associées à des champignons et des bactéries au sein d'une chaine trophique, les plantes peuvent utiliser cette forme de phosphore (Irshad et al. 2012). Jusqu'à présent, les facteurs de régulation de l'activité de la phytase ont été étudiés en conditions artificielles, où les facteurs influençant l'enzyme ont été analysés séparément et de manière contrôlée. La hiérarchisation des facteurs de contrôle de l'activité phytasique dans la rhizosphère des plantes reste à faire pour étudier les possibilités de valoriser les ressources en phytate du sol. Le projet ANR blanc UnlockP (Projet 1) a pour ambition d'étudier différentes voies d'utilisation du phytate par les plantes. Deux stratégies sont à l'étude. La première est basée sur les interactions trophiques entre un champignon mycorhizien, des bactéries et des invertébrés prédateurs (nématodes). En effet, les nématodes bactérivores en consommant des bactéries libèrent les phytases bactériennes intracellulaires dans la rhizosphère de plantes et accélèreraient la libération de P disponible à partir de phytate (Irshad et al. 2012; Irshad et al. 2011). La deuxième stratégie est de transformer le champignon mycorhizien pour lui faire libérer directement des phytases bactériennes, plus efficientes pour minéraliser le phytate dans la rhizosphère de la plante hôte. Afin d'évaluer ces stratégies, l'activité phytasique doit être intégrée dans un modèle de complexation de surface afin de tenir compte des réactions de nature géochimique contrôlant la disponibilité des ions orthophosphates libérés lors de l'hydrolyse. La grande complexité des réactions entrant en jeu dans la modification de l'activité enzymatique lors de son adsorption sur les surfaces minérales rend délicate une modélisation purement mécaniste. L'originalité des mes travaux repose sur l'analyse de l'activité enzymatique dans des sols entiers en présence d'une résine anionique ayant le rôle de puits afin de proposer un formalisme d'activité enzymatique à double compartiment. La taille de ces compartiments est fonction du pool d'enzymes libérées par chacune des stratégies. Pour cela, nous avons choisi d'évaluer l'activité phytasique de 3 phytases issue de *Aspergillus niger*, *Debaryomyces castelllii* et *Bacillus subtilis* apportée dans les sols précédemment décrits. Ces sols ont été au préalable modifié en apportant des sources de carbone assimilable, anion organique et protons afin de recréer un environnement mycorhizosphérique. Lors de ces expérimentations, nous avons dû optimiser les protocoles d'extraction du phosphore du sol à l'aide de résines échangeuses d'anions afin de ne pas perturber l'environnement chimique. Les bons résultats de cette optimisation devraient prochainement être valorisé sous la forme d'un article. La hiérarchisation des facteurs de contrôle de l'activité enzymatique est actuellement en cours à l'aide de ce protocole [C. 1]. Les premiers résultats nous montrent un effet marqué du pH et de la concentration en oxalate sur la disponibilité en Pi, avant le type d'enzymes (M2 A. Vidal, M2 C. Vetélé). Prochainement, nous mènerons des expérimentations afin d'obtenir des fonctions réponses pour chacune de ces enzymes en tenant compte des pools d'enzymes adsorbées ou libres, en commençant par le pH car c'est un facteur clef de la régulation de l'activité enzymatique via des effets directs ou indirects (Sinsabaugh et al. 2008). Une variante du protocole d'extraction par les résines anioniques a été testée au champ afin de mesurer la disponibilité environnementale de l'azote et du phosphore dans la rhizosphère de plantes seules ou en association ([C. 2], Projet 6).

Conclusions

Dans les travaux décrits dans ce premier axe, nous avons démontré la pertinence de notre démarche pour décrire et évaluer certains facteurs de la disponibilité des nutriments comme le pH. L'originalité de ces travaux réside dans l'évaluation de ce facteur dans des sols de composition minérale entièrement décrite, contrairement à des travaux contemporains n'utilisant qu'une surface absorbante (Geelhoed et al. 1998; Geelhoed et al. 1997, 1999). En condition limitante en phosphore, nous avons pu montrer, en combinaison avec les variations de pH, le rôle clef de l'adsorption sur les argiles et l'importance du prélèvement de calcium sur la disponibilité du phosphore. L'intégration des réactions biologiques bactériennes (nitrification) ou enzymatique (uréasique et phytasique) requiert la même démarche mais l'absence de formalismes mécanistes ralentit leur intégration. Une approche semi mécaniste est nécessaire et justifie une démarche pluri-disciplinaire afin de faire progresser notre compréhension.

Axe 2

Etude conjointe de la disponibilité environnementale et du fonctionnement du sol : application du concept de qualité des sols.

Introduction

Les fonctions des écosystèmes sont la résultante de processus naturels se déroulant dans les sous systèmes comme le sol. Parmi les fonctions définissant les services écosystémiques d'approvisionnement, le cycle des nutriments à partir de la matière organique dépend du fonctionnement du sol et plus précisément des interactions entre ses composantes biotiques et abiotiques (de Groot et al. 2002). Concernant les interactions biotiques, les plantes, en modifiant leur rhizosphère, peuvent affecter les réseaux trophiques du sol (Wagg et al. 2014) et contribuer à la régulation du cycle des nutriments (Sylvain and Wall 2011). Ainsi, (Wardle et al. 2004) estiment que les réseaux trophiques du sol, d'une grande complexité, seraient connectés aux réseaux trophiques aériens. Les pratiques agricoles peuvent affecter à la fois les composantes biotiques et abiotiques du sol (Pellerin et al. 2006). Quelques auteurs (Barrios 2007; Wagg et al. 2014) estiment qu'elles affectent la diversité et l'abondance des organismes du sol. Par ses pratiques, l'agriculteur peut ainsi modifier la structure et les états rédox associés ou le pH des sols ou la qualité des litières. Il est nécessaire d'évaluer les facteurs de régulation mis en avant dans l'axe 1 à l'échelle de la parcelle qui intègre l'ensemble des pratiques de l'agriculteur pour sélectionner ceux qui sont pertinents.

Démarche

La stratégie mise en œuvre dans cet axe est d'utiliser le concept de qualité des sols pour tenter de relier les variations de disponibilité environnementale des éléments avec le fonctionnement global des sols. Ce type d'approche permet en outre d'évaluer les pratiques d'intensification écologiques dans un contexte large. L'évaluation de la qualité des sols est une démarche méthodologique et un concept, introduits à la fin des années 70. Je distingue deux qualités des sols, la « qualité inhérente » et la « qualité dynamique » (Karlen 2004; Karlen et al. 2003; Karlen et al. 1997; Wienhold et al. 2004). La qualité inhérente fait référence aux propriétés naturelles et originelles des sols influencées par la roche mère, la topographie, le climat, la végétation et l'âge du sol tandis que la qualité dynamique représente l'évolution du fonctionnement du sol sous l'influence des activités humaines. L'évaluation de la qualité dynamique permet de déterminer la direction et l'intensité des effets sur le fonctionnement du sol lors d'un changement de pratique à des pas de temps relativement courts (< 10 ans), donc pertinents à l'échelle de temps humaine (Robinson et al. 2012). Le concept de qualité des sols reprend les concepts d'écologie du sol en combinant les informations données par une variété d'indicateurs. Les indicateurs peuvent être biologiques (basés sur les organismes du sol) mais également chimiques et physiques. Nous avons fait le choix de ne pas les combiner en un indicateur unique comme l'Indice de Qualité Biologique des Sols (Nuria et al. 2011) afin de ne pas perdre d'information. En effet, dans l'objectif de relier la disponibilité des éléments au fonctionnement du sol, l'utilisation de ces différents types d'indicateurs est nécessaire pour considérer la complexité du sol. L'utilisation de différents indicateurs aux temps de réponse différents après l'apparition d'une perturbation permet également d'évaluer les dynamiques à court et long terme. La démarche mise en œuvre dans ce deuxième axe de recherche s'est structurée autour de deux objectifs :

Objectif 2.1. Quels sont les indicateurs les plus pertinents pour évaluer le fonctionnement du sol et la disponibilité en nutriments ?

Objectif 2.2. Pouvons nous réellement relier le fonctionnement du sol avec les pratiques agricoles?

Afin de répondre à ces objectifs, nous avons choisi de nous intéresser aux systèmes viticoles. En effet, les sols viticoles sont particulièrement vulnérables et menacés par la perte de matière organique et de biodiversité du sol mais également l'érosion et la pollution (Coulouma et al. 2006; Hildebrandt et al. 2008; Lagacherie et al. 2006; Le Bissonnais et al. 2007). Malgré cette vulnérabilité reconnue, la prise en compte de la qualité du sol dans la définition des pratiques viticoles est restée balbutiante car cantonnée à une évaluation de la qualité inhérente. En outre, la vigne est un système pérenne. Dans ce type de système, le recyclage de la matière organique par la communauté microbienne est indispensable. Enfin, la viticulture biologique est en plein essor rendant encore plus importante la question de l'activité biologique des sols. C'est donc un modèle d'étude intéressant pour étudier l'effet des pratiques culturales sur la disponibilité en élément. Notre échelle d'étude est parcellaire, distribuées sur une région viticole à la géologie complexe. Dans ce type d'approche, des compétences associées à la collecte de données (échantillonnage, mesure) des différents indicateurs sont nécessaires mais également des compétences en analyse multivariées et univariées pour extraire l'information.

Principaux résultats

Quels sont les indicateurs les plus pertinents pour évaluer le fonctionnement des sols et la disponibilité en éléments ?

L'information portée par les indicateurs peut permettre d'identifier les actions pour améliorer ou suivre l'évolution d'un système écologique. Nous avons sélectionné un ensemble d'indicateurs permettant d'évaluer la fonction de recyclage de la matière organique et de transfert des éléments nutritifs. Des compromis dans le choix des indicateurs sont nécessaires (Culman et al. 2010). Nous avons sélectionné des indicateurs physiques, chimiques, et biologiques basés sur les nématodes, bactéries et vers de terre. Nous avons alors suivi l'évolution des valeurs mesurées sur des parcelles d'une même zone pédopaysagère dans le cas d'une conversion de la viticulture conventionnelle à la viticulture biologique. Nous avons pu montré une évolution des teneur en N total, et P, K disponible en fonction de l'âge de conversion. Nous avons formulé alors l'hypothèse que la biomasse bactérienne totale plus importante (+ 34%) ainsi qu'une plus grande abondance de nématodes fongivores (+ 97% après 17 ans de conversion) pour des conversions précoces auraient augmenté la sécrétion de ligands organiques ou de phosphatases par intensification du réseau trophique tel que mesuré par (Irshad et al. 2012; Irshad et al. 2011). L'analyse discriminante (Figure 4) basée sur les mesures des différents indicateurs (physiques, chimiques et biologiques) a montré une nette discrimination en fonction de l'âge de conversion. La viticulture biologique a conduit à une diminution significative de l'abondance et de la biomasse des vers de terre endogés (- 65 % en 17 ans). Ce résultat surprenant aurait mérité d'être complété avec la détermination des vers de terre anéciques mais nous ne pouvions pas faire cette détermination avec le nombre de parcelles étudiées. Nous avons attribué la plus faible abondance et biomasse des vers de terre endogés à la compaction des sols liée à un nombre de passages plus importants en viticulture biologique. Dans cette étude, nous avons démontré que la période de transition 7-11 ans, dépendant des indicateurs considérés, était nécessaire pour séparer clairement les pratiques conventionnelles de celles de l'agriculture biologique pour ce vignoble. [P 8].

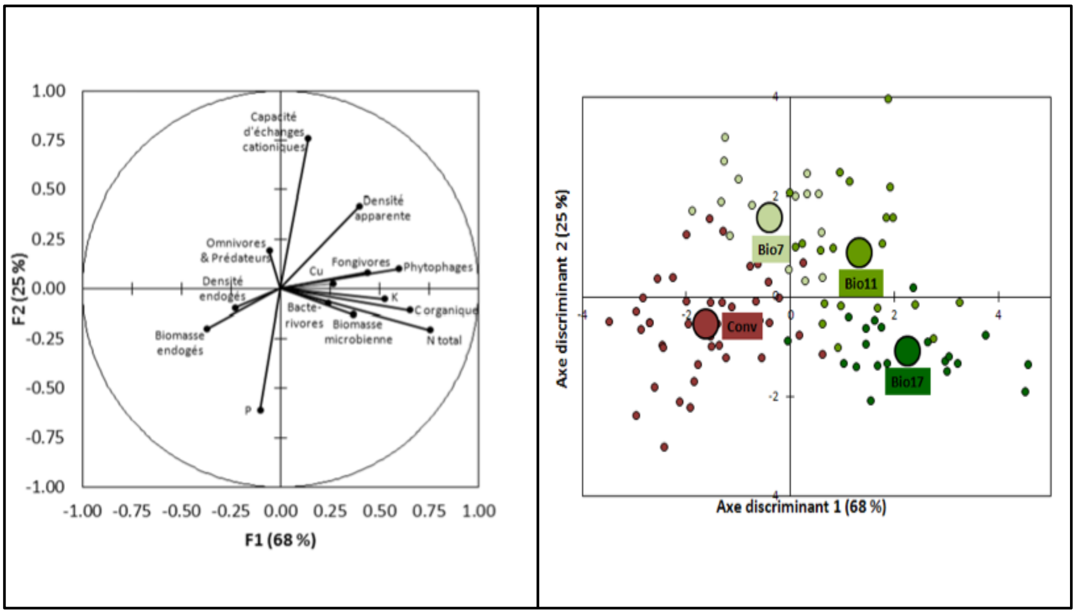


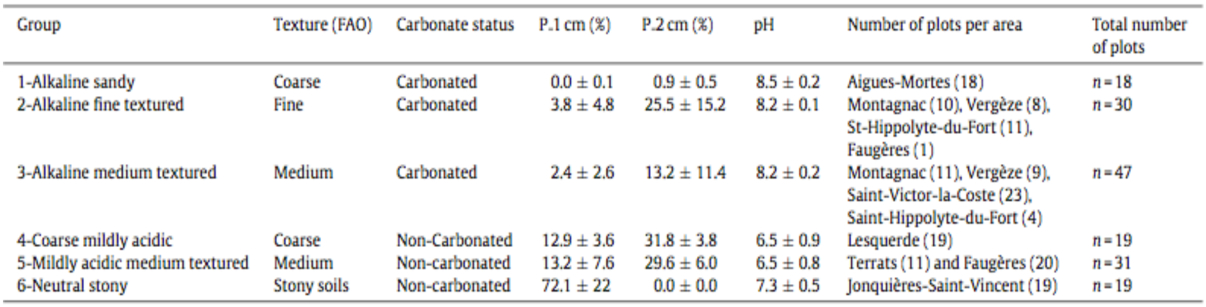
Figure 4 : Résultats de l'analyse discriminante réalisée après analyse des indicateurs avec (a) cercle de corrélations et (b) distribution des 96 observations et des centroïdes. Les termes Conv, Bio7, Bio 11 et Bio 17 renvoient à l'âge de conversion, pas de conversion (Conv), conversion depuis 7, 11 et 17 ans pour Bio7, Bio 11 et Bio 17 respectivement.

En utilisant les informations basées sur les nématodes comme l'indice d'enrichissement, nous suggérons que l'augmentation des ressources disponibles a favorisé la densité des nématodes bactérivores. Le fonctionnement du sol a été modifié avec les voies de décomposition de la matière organique devenant plus fongique que bactérienne. Même si des changements étaient observés dans la structure de la communauté de nématodes après conversion, l'indice de maturité (MI), l'indice des nématodes phytophages (PPI) et l'indice de structure (SI) sont restés constants. Par conséquent, les pratiques agricoles biologiques n'ont amélioré ni la longueur ni la complexité de la chaîne trophique du sol bien que quantitativement l'activité biologique soit plus importante. Nous concluons que le niveau de perturbations dans le sol n'a donc pas changé après conversion [P 5].

Pouvons nous réellement relier le fonctionnement du sol avec les pratiques agricoles?

L'évaluation des pratiques agricoles par l'intermédiaire des indicateurs repose sur la composition d'un référentiel qui doit tenir compter des caractéristiques naturelles des sols, les indicateurs de la qualité inhérente. Lors de la thèse de Patrice Coll, et en partie de celle de Egidio Lardo, nous avons échantillonné 164 autres parcelles réparties dans 9 zones pédopaysagères. Une première analyse nous a montré l'importance de tenir compte des propriétés inhérentes des sols comme la teneur en calcaire ou la pierrosité afin de comprendre l'effet des pratiques par type de sol. Lors du post doctorat de Clémence Salomé, nous avons utilisé cette base de données en utilisant des analyses multivariées plus spécifiques afin d'obtenir une classification opérationnelle. La classification opérationnelle est obtenue en évaluant le poids des indicateurs inhérents sur les indicateurs dynamiques. Dès lors, il a été possible de regrouper les parcelles en 6 groupes aux indicateurs inhérents proches plutôt qu'en zones pédopaysagères (Tableau 2). Des statistiques descriptives simples permettent de visualiser la variabilité des indicateurs dynamiques par groupe de sol.

Tableau 2 : classification opérationnelle utilisée pour la création du référentiel de variabilité des indicateurs de qualité dynamique. Repris de (Salome et al. 2014)



Group	Texture (FAO)	Carbonate status	P.1 cm (%)	P.2 cm (%)	pH	Number of plots per area	Total number of plots
1-Alkaline sandy	Coarse	Carbonated	0.0 ± 0.1	0.9 ± 0.5	8.5 ± 0.2	Aigues-Mortes (18)	n = 18
2-Alkaline fine textured	Fine	Carbonated	3.8 ± 4.8	25.5 ± 15.2	8.2 ± 0.1	Montagnac (10), Vergèze (8), St-Hippolyte-du-Fort (11), Faugères (1)	n = 30
3-Alkaline medium textured	Medium	Carbonated	2.4 ± 2.6	13.2 ± 11.4	8.2 ± 0.2	Montagnac (11), Vergèze (9), Saint-Victor-la-Coste (23), Saint-Hippolyte-du-Fort (4)	n = 47
4-Coarse mildly acidic	Coarse	Non-Carbonated	12.9 ± 3.6	31.8 ± 3.8	6.5 ± 0.9	Lesquerde (19)	n = 19
5-Mildly acidic medium textured	Medium	Non-carbonated	13.2 ± 7.6	29.6 ± 6.0	6.5 ± 0.8	Terrats (11) and Faugères (20)	n = 31
6-Neutral stony	Stony soils	Non-carbonated	72.1 ± 22	0.0 ± 0.0	7.3 ± 0.5	Jonquières-Saint-Vincent (19)	n = 19

La plupart des indicateurs dynamiques sont affectées par les propriétés inhérentes [P 2]. En ce qui concerne les indicateurs de disponibilité en P et K, nous n'avons pas mis en évidence d'effets significatifs du type de sol. C'est un résultat surprenant au vu des conclusions acquises dans le premier axe de recherche. La mesure de la disponibilité de ces éléments reposait sur une extraction simple à l'eau, mais des estimations avec d'autres extractants auraient été préférables. Nous avons pu souligné l'importance des propriétés inhérentes sur la gamme de variation de certains indicateurs comme ceux basés sur l'analyses des populations de nématodes, voire sur l'inadéquation d'autres comme le quotient métabolique. La prochaine étape est l'interprétation des valeurs renvoyées par les indicateurs dynamiques de la qualité des sols en fonction des pratiques. Pour cela nous avons l'objectif de tester les pratiques culturales recensées par un questionnaire (M2 Gorbyk M.). Trois types de pratiques sont testées dans un projet d'article: durée de l'enherbement, type de désherbage et le type de fertilisation (minérale ou organique). Je pense également que le développement de nouvelles techniques d'estimation de la disponibilité environnementale est indispensable. Je pense qu'avec un protocole adapté les résines échangeuses d'anions et de cations au terrain permettraient de mieux suivre les flux d'éléments dans les sols comme démontré par (Andrews and Carroll 2001). Au vu des difficultés rencontrées par la collecte de données sur un grand nombre de parcelles [P 4] et les questions autour de l'adéquation des techniques d'estimation de la disponibilité environnementale, le choix des indicateurs est l'étape critique de notre approche.

Conclusions

Notre objectif était d'estimer la disponibilité en nutriments en réponse à des modifications du fonctionnement du sol. La démarche mise en œuvre ici est originale car à notre connaissance des travaux pionniers car associant des d'indicateurs physico-chimiques et biologiques, indicateurs biologiques incluant la microfaune (nématodes) et la macrofaune (vers de terre) pour l'étude de sols viticoles. Cette approche écologique du fonctionnement du sol permet d'évaluer l'intensité des modifications de disponibilité des éléments. Contrairement à (Gil-Sotres et al. 2005), nous estimons que l'utilisation d'indicateurs physico-chimiques est pertinente car ces indicateurs réagissent aux modifications de pratiques agricoles à condition d'analyser les informations renvoyées par les indicateurs par groupe de sols aux qualités inhérentes proches. Nous essaierons dans un prochain article de relier les pratiques culturales aux modifications du fonctionnement du sol par groupe de sols. Par manque de temps, d'argent et de compétences, nous n'avons pris en compte que la biomasse microbienne (indicateur biochimique général) mais pas les activités enzymatiques (indicateur biochimique spécifique) comme suggéré par (Gil-Sotres et al. 2005; Trasar-Cepeda et al. 2008). Or, il aurait été pertinent de les utiliser pour comprendre les différences de teneurs en éléments disponibles car nous supposons des différences d'activités enzymatiques sous différentes pratiques agricoles. Cette complémentarité d'approche peut guider l'usage des terres et le choix des pratiques culturales promouvant l'ensemble des fonctions du sol impliquées dans différents services écosystémiques (Andrews and Carroll 2001). Nous avons pu identifier quelques indicateurs pertinents pour étudier les modifications du fonctionnement du sol mais nous n'avons pas été au bout de notre démarche car l'étude de la disponibilité en nutriments par les méthodes employées n'était pas satisfaisante. La mise au point méthodologique sur les résines échangeuses d'ions et leur application au champ est prometteuse pour mieux suivre les flux d'éléments dans les sols et les relier aux changements du fonctionnement du sol (voir axe 3). Notre expertise acquise sur cet axe peut être utilisée pour mieux comprendre les cycles des éléments après fertilisation organique ou lors des associations de culture afin de promouvoir la qualité des sols et maintenir les fonctions du sol impliquées dans les services écosystémiques. La richesse de la base de données nous permet à l'avenir de comparer le fonctionnement des sols dans une culture pérenne par rapport à des cultures annuelles.

Axe 3

Recherche d'applicabilité des travaux

Avertissement : le contenu de cette partie comporte des informations confidentielles à ne pas divulguer

Réflexions sur l’activité d’encadrement et de recherche

La rédaction de ce document est l'occasion de faire une introspection sur ma trajectoire et de l'analyser. L'Habilitation à Diriger les Recherches, qu'est ce en fin de compte ? C'est un diplôme sanctionnant une formation d'encadrant de jeunes chercheurs grâce à la définition d'une stratégie de recherche suffisamment large et originale (*Arrêté du 23 novembre 1988 relatif à l'habilitation à diriger des recherches).* Je vais m'arrêter ici sur les différents points de cette définition et faire l'analyse de mes travaux sur la base des fiches synthétiques (décrites en annexe) de mes encadrements et du bilan de publication. Le doctorat est une formation mais je pense qu'il faudrait parler d'accompagnement scientifique et personnel plutôt que d'encadrement. Je pense que notre rôle d'encadrant repose sur l'écoute, sur le respect des intérêts scientifiques et personnels de l'étudiant, et un encouragement de la levée de l'autocensure des étudiants. Je pense qu'il faut favoriser la créativité des étudiants en les rassurant sur leurs capacités tout en les aidant à s'organiser et à argumenter leurs choix. C'est pour toutes ces raisons que je parlerai d'accompagnement plutôt que d'encadrement.

Le métier d'enseignant chercheur donne un accès privilégié aux étudiants pour présenter les thématiques de recherche. Parmi les étudiants que j'ai eu en co-encadrement de thèse (n=4), 2 sont des étudiants issus de formations recherche (M2 Ecosystèmes N. Devau) ou ingénieurs (P. Coll) dans lesquelles je suis intervenue. Ces deux étudiants ont obtenu un CDI dans des structures de recherche publiques (BRGM, Nicolas Devau) ou privé (laboratoire Rière, Patrice Coll) conformément à leur projet professionnel. Je résume dans le tableau ci dessous les principales données des fiches synthétiques (voir annexe) autre que les publications renseignées dans le Tableau 1.

Tableau 3 : description des valorisations des étudiants. Les étudiants en vert ont obtenu un CDI, en orange un contrat à durée déterminée, en rouge sont en recherche d'emploi.

Doctorant	Année début thèse	durée thèse	Type de financement de la bourse	Origine du M2	Nb articles ACL 1er auteur	Nb articles ACL co auteur	Nb communications orales internationales avec actes et comité de lecture	Nb rédaction vulgarisation	Dépôts INPI
Trouillefou C	2010	3,5	CIFRE	Master Matériaux Université de Aix Marseille	1	0	0	0	1 2 en cours
Coll P	2008	3	ADEME/MSA	Ingénieur agronomie Spécialité Viticulture oenologie	2	2	2	2	0
Lardo E	2008	3	bourse ministérielle (Italie)	International Ph.D. Programme «Crop Systems, Forestry and Environmental Sciences	1	1	0	0	0
Devau N	2007	3	INRA/Région	M2 recherche UM2 Fonctionnement des écosystèmes naturels et Cultivés (ECOSYSTEMES)	4	1	2	0	0

J'ai systématiquement proposé à mes étudiants de participer à des enseignements de deuxième année et troisième année d'école et en master recherche. C'est une autre façon de valoriser les résultats de la recherche et de former des jeunes en école d'ingénieur ou à l'université.

Mes travaux contribuent à une réintégration de la composante biologique du sol autre que le peuplement cultivé dans les agro-écosystèmes. Le rapprochement entre l'écologie des sols et les sciences de la terre (géochimie) permet de reprendre les questions de recherche sur les flux minéraux de considérer de nouvelles échelles d'action et d'évaluation des pratiques agricoles. L'originalité de mes travaux de recherche porte sur l'approche pluri disciplinaire de la disponibilité des éléments et de la diversité des méthodes employées ainsi que des échelles d'études (rhizosphère à parcelle agricole). La pluridisciplinarité est enrichissante car elle peut renouveler nos concepts et approches. La pluridisciplinarité est en outre intéressante à développer dans les enseignements d'école d'ingénieur agronome. Je développe une stratégie dans la partie projet en réponse aux limites de mes précédents travaux à savoir (i) la prise en compte faible des activités enzymatiques dans la rhizosphère des plantes et (ii) la pauvreté des relations entre pratiques agro écologiques et types de sol. J'ai également la conviction que mes travaux doivent se rapprocher de l'écophysiologie des plantes pour tenir compte des effets d'adaptation des besoins de la plante à la disponibilité en nutriments.

Le financement de mes travaux s'est réalisé en partie *via* des collaborations de recherche avec des partenaires industriels. Les discussions avec les directions « recherche et développement » d'entreprises permettent de sensibiliser ces professionnels aux problématiques actuelles des cycles biogéochimiques et d'envisager avec eux la préservation des services écosystémiques. L'expérience de collaborations de recherche avec les différentes partenaires industriels est satisfaisante car ce sont des entreprises de taille importante avec un secteur recherche-développement constitué en partie de docteurs, donc de personnes habituées à la démarche. Je maintiendrai donc ce lien car outre, les aspects scientifiques, c'est un vivier de recrutement potentiels des doctorants.

.

Projet scientifique

Je propose un projet de recherche afin de à (i) lever les verrous de la prise en compte de la biologie des sols pour comprendre la disponibilité des éléments nutritifs et à (ii) de mieux tenir compte de l'écophysiologie de la plante pour tenir compte des effets d'adaptation de la plante aux conditions limitantes en nutriments.

Le premier objectif est composés de deux étapes. A court terme, je propose d'identifier et de modéliser l'activité enzymatique de la rhizosphère. A moyen terme, je souhaite intégrer ma réflexion dans des études sur la compréhension du fonctionnement du sol. Enfin dans une perspective à plus long terme, je souhaite développer des liens avec l'écophysiologie de la plante, je propose alors quelques pistes de travail.

QUELS SONT LES VERROUS DE LA MODELISATION DE L'ACTIVITE ENZYMATIQUE DANS LA RHIZOSPHERE ?

Je fais le bilan d'une nécessaire prise en compte des effets de rétro-contrôle entre les composantes biotiques et les facteurs abiotiques du milieu pour mieux comprendre et prédire la disponibilité des éléments. La modélisation mécaniste géochimique peut être utile pour comprendre la disponibilité des éléments minéraux à condition de tenir compte des activités biologiques dans la rhizosphère.

Ma contribution à la question générique de recherche de compréhension de la disponibilité des éléments en condition de bas intrants est l'évaluation de l'activité des enzymes extracellulaires dans la rhizosphère des plantes. L'élément modèle sera le phosphore, et plus particulièrement le phytate. Je rappelle que la fraction organique de phosphore dans les sols peut être largement majoritaire mais que les plantes seules n'y ont que faiblement accès en raison de leur capacité limitée à secréter des enzymes alors que les bactéries et champignons peuvent excréter de grandes quantités d'enzymes (phytases) susceptibles d'hydrolyser les liaisons esters permettant de libérer l'ion orthophosphate à partir de molécules de phosphore organique. L'activité des enzymes extracellulaires libérées par les organismes au sein de la rhizosphère est donc impliquée dans la disponibilité des nutriments, mais pas intégrées dans les modèles géochimiques. En outre, les enzymes extracellulaires peuvent être considérées comme un marqueur des activités microbiennes modifiées par les changements physiologiques de la plante en réponse à l'augmentation de la pCO_2 atmosphérique (Burns et al. 2013). Outre leur implication dans la nutrition des plantes, la compréhension de leur régulation pourrait contribuer aux connaissances sur les mécanismes de stockage de carbone, et de résilience des agro écosystèmes aux changements climatiques. Quelque soit la nature de l'organisme producteur, les enzymes secrétées, ou libérées dans le milieu par lyse sont susceptibles d'interagir avec les constituants solides du milieu et les autres facteurs abiotiques (température, teneur en eau, pH…). Dans un premier temps, je pense qu'il faut se concentrer sur ces facteurs abiotiques, les mécanismes de libération des enzymes étant étudiés par ailleurs dans l'UMR Eco&Sols suite à l'ajout de litières végétales (I. Bertrand). Nous pourrons établir des liens entre nos travaux.

La rhizosphère est une zone de sol modifiée par l'activité des racines, elle est donc dynamique (Carminati 2012). Parmi les facteurs susceptibles de modifier l'activité des enzymes extracellulaires (Figure 10), le pH, la teneur en eau ou la modification des propriétés de surface des colloïdes du sol par les composés exsudés par les racines sont particulièrement importants. Il existe des travaux sur la modélisation de l'activité enzymatique dans les sols mais

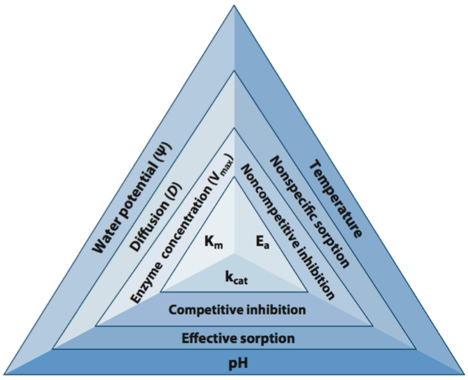


Figure 10 : représentation du contrôle environnemental des enzymes libérées dans le milieu. Les cinétiques enzymatiques sont représentées par les paramètres k_{cat} (vitesse de conversion du substrat), K_m (constante de Michaelis Menten), and E_a (énergie d'activation), repris de (Burns et al. 2013)

déconnectés de la régulation réaliste des facteurs abiotiques sur celle ci (Steinweg et al. 2012), et particulièrement pour le processus d'adsorption délicat à prédire dans un sol (George et al. 2007a; Kedi et al. 2013a; Kedi et al. 2013b; Louche et al. 2010) .

Mon hypothèse de recherche est que l'évaluation de l'activité enzymatique des sols rhizosphériques nécessite des fonctions réponses adaptées à ce compartiment de sol à cause des modifications des propriétés du sol induites par les activités racinaires.

Stratégie de recherche

Pour appréhender la complexité du système que j'étudie, je privilégierai une approche expérimentale, simplificatrice et réductionniste en vue de la modélisation. L'approche que j'envisage est principalement empirique dans un premier temps afin d'établir des fonctions réponses de l'activité enzymatique extracellulaire dans la rhizosphère dans l'objectif d'intégration de l'activité enzymatique dans les modèles de géochimie. Je développerai une approche basée en 3 étapes:

Etape 1. Identification des gammes de variations de l'activité phytasique pour des sols mycorhizophériques artificiels, et identification des facteurs prépondérants (Projet 1)

Les sols mycorhizosphériques sont les sols modèles de l'UMR (Chromic Cambisol de Cazevieille et Luvisol de Auzeville), non stériles, dont l'ambiance géochimique aura été modifiée par l'ajout de molécules secrétées par les plantes ou les champignons et bactéries associées. L'identification et les proportions sont déterminées à partir de la littérature On peut citer en exemple l'oxalate ou carbone assimilable. A ces sols « mycorhizosphériques », je rajouterai des enzymes exogènes (Projet 1) de *Aspergillus niger, Bacillus subtilis* et *Debaryomyces castelli* comme enzymes modèles. En fonction de la gamme de réponse obtenue dans les plans expérimentaux, je sélectionnerai les facteurs les plus influents et évaluerai la proportion d'enzymes adsorbées *versus* libre. En parallèle de ce travail, j'évaluerai la proportion d'enzymes intra cellulaires et extracellulaires par extraction de sol dans une expérimentation menée par mes collègues J. Trap et C. Plassard en microcosmes constitués d'une plante mycorhizée (Pin maritime *Pinus pinaster* et *Hebeloma cylindrosporum*), d'un nématode (*Rhabditis sp*)et d'une bactérie (Bacillus subtilis) en présence de phytate. Ces résultats pourront être également confrontés à ceux d'autres collègues impliqués dans le projet (H. Quiquampoix, H. Le Guernervé) qui utiliseront la RMN 2D DOSY pour étudier l'interaction des enzymes à differents pH.

Etape 2. Obtention de formalismes réponses de l'activité enzymatique.

L'approche expérimentale de (Steinweg et al. 2012) est particulièrement intéressante pour obtenir des formalismes de réponse comme ceux recherchés dans mon projet. Ces auteurs ont obtenu des formalismes de réponse de type Q_{10} pour la température ou vis à vis de la teneur en eau. Brièvement, il s'agit de mesurer l'activité enzymatique en microplaque (96 puits) à l'aide d'un spectrofluorimètre. Dans le cas du projet UnlockP, un substrat fluorescent analogue au phytate est en cours d'obtention. La technique en microplaque utilisant peu de substrats (sol et phytate), elle serait donc pertinente pour obtenir ces fonctions réponses. En outre, elle permettrait par la suite de recueillir du sol rhizosphérique et d'analyser si ces fonctions réponses sont valides afin de scénariser l'activité enzymatique sur le terrain si on connaît la variabilité temporelle au champ de certains facteurs comme le pH ou la température.

Les étapes 1 et 2 seront faites principalement sur financement du projet ANR de Claude Plassard et en collaboration avec les partenaires impliqués dans le projet. Le cas échéant, si l'ensemble des résultats n'est pas acquis dans le cadre de ce projet, je pense solliciter nos partenaires industriels, TIMAC agro international via le montage d'une ANR de type recherche collaborative.

Etape 3 : vers une modélisation plus mécaniste de l'activité enzymatique

J'analyserai ensuite les formalismes de la bioacatalyse supportée développés par les chimistes des matériaux. L'école de chimie de Montpellier, et plus particulièrement l'équipe MACS (Matériaux Avancés pour la Catalyse et la Santé) et l'équipe « Matériaux et Biologie » du Laboratoire de la Chimie de la Matière Condensée de Paris (Université Pierre et Marie Curie) seraient des interlocuteurs avec qui je compte collaborer. En effet, ces équipes ont des compétences en modélisation physico-chimique des enzymes supportées. Il est frappant de constater les analogies entre le fonctionnement des enzymes supportées et celles du sol car certains processus influençant l'activité comme l'adsorption ou le piégeage dans le réseau poral sont communs . Il est donc tentant d'examiner les formalismes des enzymes supportées pour voir si ceux ci ne pourraient être adaptés aux sols.

OUVERTURE DE MA THEMATIQUE DE RECHERCHES VERS D'AUTRES QUESTIONS DE RECHERCHES

… pour mieux comprendre le fonctionnement du sol et des services écosystémiques

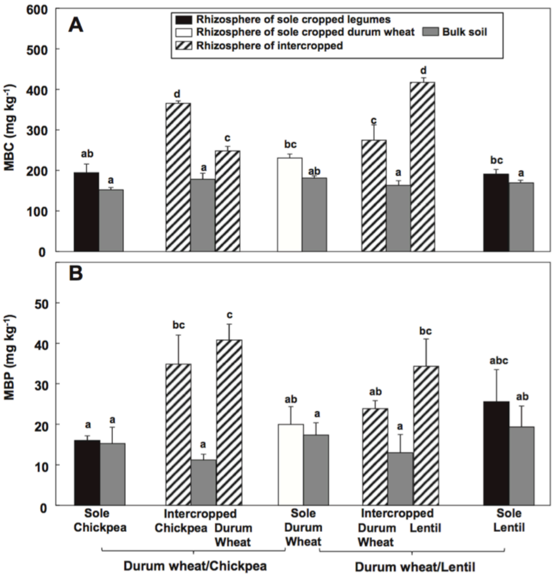


Figure 11 : Teneur en Carbone (A), et phosphore (B) dans la rhizosphere et le sol non rhizosphérique de blé, pois chiche et lentille cultivés seuls ou en association, repris de (Tang et al. 2014)

Dans un cadre plus général de compréhension du fonctionnement du sol et des services associés, l'évaluation des systèmes de culture à base d'itinéraires techniques relevant de l'agroécologie comme les associations de culture doit être réalisée (Brooker et al. 2014). (Geisseler and Scow 2014) montrent que les applications répétées d'engrais azotés peuvent altérer la composition des communautés microbiennes en utilisant différents indicateurs microbiens et biochimiques. Ces auteurs insistent sur la cinétique lente des changements et donc l'importance des suivis à des pas de temps supérieurs à 2 ou 3 ans. Dans une de nos études, nous avons déterminé une période de 7-11 ans [P 8]. Les nouvelles pratiques agroécologiques doivent être donc être évaluées à une échelle de temps de plusieurs années pour analyser leurs effets globaux sur le fonctionnement des écosystèmes et pas seulement comme alternative à une réduction des intrants. Récemment, (Tang et al. 2014) ont testé l'hypothèse que l'association entre des plantes peut avoir un effet sur la communauté bactérienne et le cycle du P. Ces auteurs montrent que la teneur en C, N et P de la biomasse microbienne de la rhizosphère est plus importante dans les cultures associées que pures (Figure 11**Erreur ! Source du renvoi introuvable.**). Lors d'un projet avec un collègue de mon UMR (Louis Mareschal), nous avons testé si l'association entre plantes permettait la facilitation d'un élément comme le P. Nous avons mis en évidence des variations de la disponibilité en N et P en fonction de *scenarii* contrastés de gestions sylvicoles au Congo et au Brésil dans des associations de culture avec des légumineuses (Projet 6, [C. 2]). Par contre, dans des associations céréales-légumineuses en France, nous n'avons pas mis en évidence de différences de disponibilités en P, seulement un effet du précédent cultural. D'après (Brooker et al. 2014), les études sur les pratiques agro écologiques comme les cultures associées se focalisent sur un processus plutôt que sur les multiples interactions entre processus. Par conséquent, il est délicat d'identifier les limitations des processus majeurs comme la disponibilité en nutriments limitant les rendements ou les services écosystémiques. Dans leur synthèse sur les cultures associés, (Brooker et al. 2014) insistent également sur la nécessité d'étudier la santé du sol (concept proche de la qualité des sols) dans le cas des cultures associées. Selon ces auteurs, les bénéfices des cultures associées peuvent être type de sol dépendantes. Ce résultat étayé par nos travaux ([P 2][P 5][P 8]) sur l'importance de la qualité inhérente des sols me questionne sur l'adaptation des pratiques agro écologiques en fonction du type de sol. Ma question de recherche est la suivante : existe t'il un potentiel d'adaptation des pratiques agro écologiques en fonction du type de sol ?

Stratégie de recherche

Je pense que l'utilisation des indicateurs de fonctionnement des sols intégrant les activités enzymatiques, associées au suivi de la dynamique des flux au sein de la rhizosphère et résidusphère au moyen de résines échangeuses d'ions placées au champ peut permettre d'évaluer les pratiques agro écologiques. Cette stratégie couplée à un utilisation efficace des outils statistiques peut nous faire avancer dans notre compréhension du fonctionnement du sol (qualité des sols) et des services écosystémiques associés. Notre méthodologie a démontré son efficacité sur l'étude de la qualité des sols et la disponibilité en nutriments. Je souhaiterai établir en fonction d'un gradient latitudinal, un réseau d'expériences de pratiques agro écologiques afin de tester ma question de recherche. Pour ce faire, outre les compétences internes de l'UMR, et ses sites expérimentaux comprenant des cultures associées pérennes ou annuelles, je pourrai me tourner vers des unités comme le Centre d'Ecologie Fonctionnelle et Evolutive de Montpellier, l'UMR d'Agroécologie de Dijon pour compléter nos indicateurs notamment de trait fonctionnels des plantes ou de biologie moléculaire. Typiquement, ce type de projet pourrait être financé par des programmes internationaux (ANR international) ou d'échanges bilatéraux.

DES ENVIES, DES IDEES POUR LE FUTUR

Cette partie n'est pas intégrée dans les perspectives à court et moyen terme de mon projet de recherche mais j'aimerai étudier ces questions par des discussions avec des collègues écophysiologistes. J'ai deux idées qui pourraient être partagées.

Idée 1. Les changements climatiques intègrent l'augmentation de la pCO_2 et ses effets sur la physiologie de la plante et par conséquent son exsudation (Jossi et al. 2006). En conditions de bas intrants, la plante peut adapter sa physiologie afin de conserver ses nutriments. Par exemple, le phénomène de résorption est connu pour influencer la dynamique des éléments en modifiant la qualité des litières (Vergutz et al. 2012). L'intensité de la résorption serait fonction de la disponibilité en éléments dans le sol (Wang et al. 2014). En conditions de bas intrants, la résorption des éléments peut elle contribuer à limiter la disponibilité des éléments ?

Idée 2. Certains auteurs montrent un lien entre la nutrition ammoniacale et potassique (Balkos et al.) On peut donc légitimement se demander comment le potassium en contrôlant les flux d'eau et d'éléments vers la plante (mass flow et diffusion) contrôlera en retour la disponibilité des éléments N et P pour les organismes de la rhizosphère des plantes et les plantes. Par conséquent, je me pose la question du contrôle de la disponibilité en N et P par le K en contexte de changement climatique.

J'exprime dans cette partie une envie d'intégrer des aspects d'écophysiologie dans mon projet de recherche, je pense rencontrer en premier lieu vers mes collègues écophysiologistes de l'UMR mais également les collègues montpelliérains du LEPSE (Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux).

CONCLUSIONS

C'est avec enthousiasme que j'ai terminé la rédaction de cette partie « Projet scientifique » de mon dossier de candidature d'HDR. Mes travaux de recherche et le projet que je souhaite développer ont pour ambition de mieux comprendre la disponibilité des éléments dans les écosystèmes terrestres. Mes approches tentent de concilier la géochimie avec l'écologie des sols afin de mieux comprendre le rôle des végétaux dans les cycles biogéochimiques. L'intérêt d'un tel rapprochement est crucial pour l'enseignement en école d'agronomie et au cœur des problématiques de l'INRA pour évaluer, et proposer des pistes de pratiques agro écologiques.

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Annexes

Annexe 1 : fiches synthétiques des encadrements

FICHE SYNTHETIQUE THESE DE DOCTORAT DE CHRISTOPHE TROUILLEFOU

2010-2014

Formation initiale : M1 en Master Matériaux Minces ou Divisés, Université de Provence, Marseille, M1 (2009-2010) Management de l'Environnement, Valorisation et Analyse, Université Paul Cézanne, Marseille, L3 (2008) Génie de l'Environnement et aménagement, Université de Provence, IUP DENTES, Marseille

M2 : master matériaux, université de Provence Aix Marseille 1.

Financement : bourse CIFRE et fonctionnement sur collaboration de recherche (Projet 2).

Encadrement: 1 directeur de thèse (C. Plassard, DR2 INRA HDR, UMR Eco&Sols), 1 co directeur (E. Belamie, MC EPHE, Institut Claude Gerhardt), 1 co directrice (E. Le Cadre, MC Montpellier SupAgro)
.
Valorisation des travaux: Christophe Trouillefou a valorisé sa thèse dans 1 article ACL, 1 enveloppe Soleau et 2 brevets en cours de rédaction. Christophe Trouillefou est intervenu pour effectuer des TD de 1ère année d'école en thermodynamique des réactions et des transferts.

Historique du projet et insertion professionnelle de l'étudiant

Lors d'une précédente collaboration (stage M1 P. Collet), j'avais rencontré E. Belamie et discuté avec lui des potentialités des matériaux mésoporeux. Lors d'une discussion informelle avec la direction R&D de Timac Agro international, mon employeur avant mon recrutement à Montpellier SupAgro, nous nous sommes convaincus de la nécessité de concevoir de nouveaux engrais qui accompagneront les pratiques agro écologiques des agriculteurs. L'idée d'un engrais à base de matériaux mésoporeux est née mais pour le mener à bien il fallait que je m'entoure de E. Belamie et Claude Plassard pour accompagner de projet. Bien que nous soyons 3 co encadrants, notre organisation, compréhension mutuelle des contraintes d'emploi du temps respectives, et respect des compétences de chaque ont permis d'encadrer efficacement C. Trouillefou pour faire la preuve de notre concept. C. Trouillefou avait en outre le profil et le tempérament adéquat pour ce type de sujet très exploratoire, et entre deux disciplines.

Bilan personnel :

Je retiens de cette thèse que l'approche multi disciplinaire est une réelle source d'innovation et que nous sommes peut être trop frileux pour oser aller vers des disciplines éloignées des nôtres. Je pense que les succès rencontrés lors de cette thèse sont aussi dus à l'entente entre les encadrants, l'écoute et le respect mutuel, mais aussi la solidarité pour que Christophe ait toujours un contact possible lors des périodes d'emploi du temps saisonnièrement chargées pour E. Belamie et moi.

FICHE SYNTHETIQUE POST DOCTORAT DE CLEMENCE SALOME

2012-2013

Formation initiale : M1 Master Biologie, Géosciences, Agroressources et Environnement de l'Université des Sciences et Techniques Université Montpellier 2, Spécialité Ecologie Fonctionnelle et Développement Durable, parcours Fonctionnement des Ecosystèmes Naturels et Cultivés (FENEC)
M2 : Sol: Organisation, Fonctionnement Gestion" de la spécialité "Environnements continentaux et hydrosciences" de la mention SAGEP: "Sciences Agronomiques, Environnement et Paysage" du Master "Sciences et technologies du vivant, AgroParisTech

Thèse : université de Neuchatel, Suisse

Financement :.collaboration de recherche avec Timac Agro International (Projet 3)

Valorisation des travaux : 1 publication et 1 en préparation, 1 journée de formation auprès de la division recherche et développement de Timac Agro International, 1 sortie terrain avec les 2ème année d'école (spécialité viticulture œnologie) et master vigne et vin.

Historique du projet et insertion professionnelle de l'étudiant : à l'issue de la thèse de Patrice Coll, j'ai souhaité continuer la valorisation de la base de données acquises. J'ai rencontré la direction de Timac Agro International à qui j'ai exposé le projet. Convaincus par la nécessité d'une meilleure formation de leurs technico commerciaux sur le fonctionnement du sol, et par le souhait de se développer sur les régions viticoles, le directeur R&D a accepté de financer une année post doctorale pour extraire l'information de la base de données. A l'issu de son année post doctorale, Clémence a choisi de ne pas continuer dans la recherche pour se tourner vers le conseil agronomique et la formation en Suisse où réside son conjoint.

Bilan personnel : c'est ma première expérience d'encadrement post doctoral. J'ai apprécié de travailler avec un jeune chercheur dont les compétences différentes des miennes m'ont permis de progresser scientifiquement. A la différence d'un étudiant en doctorat, la personne est formée à la démarche scientifique et on attend de cette personne de prendre en charge le projet. C'est ce qu'a fait Clémence avec de grandes qualités scientifiques mais également humaines. J'ai apprécié ses qualités pédagogiques lors d'une sortie terrain avec mes étudiants de la spécialité viticulture-œnologie.

FICHE SYNTHETIQUE THESE DE DOCTORAT DE PATRICE COLL

2008-2011

Formation initiale : IUT, ingénieur agronome, spécialité Viticulture-œnologie Montpellier SupAgro.
M2 : ingénieur agronome, spécialité Viticulture-œnologie Montpellier SupAgro.

Financement : bourse Montpellier SupAgro/ADEME, et financement projet AIDY (Projet 5) et AO interne UMR Eco&Sols (Projet 7).

Encadrement: 1 directeur de thèse (C. Villenave, CR1 IRD HDR, UMR Eco&Sols), 1 co directrice (E. Le Cadre, MC Montpellier SupAgro, UMR Eco&Sols)

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Valorisation des travaux: Patrice Coll a publié 2 articles en premier auteur et 2 articles en co auteur. Il a communiqué ses résultats oralement dans 2 conférences internationales avec actes, et vulgarisé ses travaux dans différents supports. Patrice Coll a participé aux Unités d'Enseignement que je coordonne : UE géochimie et écologie du sol en 2ème année du Tronc Commun de la formation ingénieur Montpellier SupAgro.

Historique du projet et insertion professionnelle de l'étudiant

Je connais Patrice depuis sa rentrée en première année à Montpellier SupAgro. J' ai interagi avec lui plus particulièrement lors de sa spécialisation en viticulture œnologie. Fils de viticulteur, Patrice était intéressé par le fonctionnement des sols viticoles. Nos échanges ont abouti sur la nécessité d'une meilleure compréhension du fonctionnement des sols viticoles. Ainsi, nous lui avons proposé un stage de fin d'études d'ingénieur qui portait sur l'évaluation du fonctionnement du sol sous vigne associée ou non à un enherbement. Les résultats de son M2 nous ont incité à demander une bourse de thèse à l'ADEME et à Montpellier SupAgro que nous avons obtenu. Immédiatement après sa thèse, il a été recruté dans un bureau d'études, conformément à son projet professionnel défini lors du Nouveau Chapitre de la Thèse. Patrice a su mobiliser ses connaissances et en acquérir de nouvelles comme les statistiques multivariées. Nous sommes restés en contact personnellement et professionnellement car nous étudions la possibilité de valoriser les spectres NIRS/MIRS acquis sur ses échantillons de thèse.

Bilan personnel :

C'est la deuxième thèse que j'ai co encadrée, 3 ans après mon arrivée dans l'UMR, et qui a débuté un an après celle de Nicolas Devau. La viticulture œnologie est une des spécialités de Montpellier SupAgro dans laquelle j'interviens. Ce contact privilégié avec les étudiants m'a permis de repérer Patrice et de définir avec lui un sujet de thèse correspondant à nos attentes scientifiques respectives. Les qualités d'organisation et d'autonomie mais également une formation adaptée et des relations avec le monde viticole, ont permis à Patrice de prendre en main son sujet. Lors de la thèse de Patrice, nous avons associé un Projet d'Elève Ingénieur pour l'étude de la perception de la qualité des sols par les viticulteurs, et organisé une sortie (pluvieuse) sur le terrain avec les étudiants de la spécialité Viticulture Œnologie et du master Vigne et Vin pour obtenir des données. Le personnel de l'UMR était également présent. Je pense pouvoir dire que c'était un bon moment de rencontre entre l'UMR et les étudiants de l'école et pour moi une façon différente de faire des liens. Les étudiants ont ensuite élaboré une fiche de synthèse des résultats qui a été jugée par l'UMR et reprise en notation par les enseignants. Le soutien de l'UMR a été très important à la fois sur le plan financier que par l'implication des personnes dans la collecte de données. Je garde un excellent souvenir de cette thèse car je me suis sentie encadrante à part entière et ai beaucoup appris au contact de Patrice et des viticulteurs.

FICHE SYNTHETIQUE THESE DE DOCTORAT DE NICOLAS DEVAU

2007-2010

Formation initiale : Université Montpellier 2
M2 : Master Biologie, Géosciences, Agroressources et Environnement de l'Université des Sciences et Techniques Université Montpellier 2, Spécialité Ecologie Fonctionnelle et Développement Durable, parcours Fonctionnement des Ecosystèmes Naturels et Cultivés (FENEC)

Financement : bourse INRA/Région Languedoc Roussillon et ANR ANR Systerra Perfcom Peuplements complexes performants en agriculture bas intrants – Interaction multitrophiques et facilitation intergénotypiques.

Encadrement: 1 directeur de thèse (F. Gérard, CR1 INRA, UMR Eco&Sols), 1 co directrice (E. Le Cadre, MC Montpellier SupAgro, UMR Eco&Sols) et 1 co encadrant de thèse (P. Hinsinger, DR1 INRA, UMR Eco&Sols)

Valorisation des travaux de thèse : Nicolas Devau a publié 5 articles en premier auteur dans des revues internationales à comité de lecture (Devau et al. 2011a; Devau et al. 2011b; Devau et al. 2010a; Devau et al. 2009; Hinsinger et al. 2011c) à l'issu de sa thèse et a participé à deux conférences internationales avec actes (Devau et al. 2008; Devau et al. 2010b). Nicolas Devau a participé à l'unité d'enseignement optionnelle que je coordonne : UE géochimie et écologie du sol en 2ème année du Tronc Commun de la formation ingénieur Montpellier SupAgro.

Historique du projet et insertion professionnelle de l'étudiant
J'ai rencontré Nicolas Devau lors du stage d'immersion de la promotion FENEC 2006-2007 que je co-encadrais avec Benoit Jaillard (DR INRA). Nos discussions sur l'importance de la géochimie dans la compréhension de la disponibilité des nutriments ont motivé cet écologue à faire un virage thématique. Jeune recrue dans l'UMR Eco&Sols, je me suis entourée de mes collègues F. Gérard et P. Hinsinger pour les aspects géochimiques et fonctionnement de la rhizosphère. Ma contribution dans cette thèse est l'approche expérimentale pour étudier l'effet des variations du pH et la co définition du sujet de M2 et du sujet de thèse.

A l'issu de cette thèse, Nicolas Devau a obtenu un poste de chercheur au BRGM.

Bilan personnel :
Le M2 et la thèse de Nicolas Devau se sont déroulés immédiatement après mon recrutement à Montpellier SupAgro. Cette période a été chargée pour moi car coïncidant avec une préparation de cours nouveaux et parfois éloignés de ma formation et la mise en œuvre d'un projet de recherche dans une UMR en reconstruction (fusion d' UMR et d'UR). Le co encadrement était nécessaire pour mener à bien ce travail dans ce contexte. J'ai retenu de cette expérience que le co encadrement avec des chercheurs seniors bien que profitable nécessite une organisation et une communication définies dès le début de la thèse pour faire coïncider activité d'enseignements et de recherche, et clarifier les rôles respectifs auprès de l'étudiant. Cette thèse m'a permis de définir rapidement mon projet de recherche et de progresser rapidement en géochimie.

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ORIGINAL PAPER

Protected activity of a phytase immobilized in mesoporous silica with benefits to plant phosphorus nutrition

Christophe Marcel Trouillefou · Edith Le Cadre · Thomas Cacciaguerra · Frédérique Cunin · Claude Plassard · Emmanuel Belamie

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Abstract Phosphorus (P) is one of the main nutrients required for plant growth and is absorbed and metabolized in the form of orthophosphate ions (Pi). In agriculture, P is a major component of fertilizers. Forecast shortages in P mine stocks within the coming decades call for alternative sources of this element for agricultural use. In the present study, we explore the possibility of incorporating phytase near the plant root system. Phytase is an enzyme capable of mobilizing the main organic form of P as phytate, which can represent up to 90 % of the total P stock in soils. We report the immobilization of phytase in KIT-6 silica mesoporous materials with large pores of 8.6 nm. The enzymatic activity is localized in the porous network, and the main kinetic features of the free phytase appear to be maintained within the functional material ($pH_{opt} = 5$–5.5 and $T_{opt} = 55$ °C). Most importantly, the enzyme immobilized in the pores shows a higher temperature stability and appears to be protected from protease degradation. Finally, phytase-loaded KIT-6 proved efficient at hydrolyzing phytate and delivering inorganic P to growing *Medicago truncatula* plants, which accumulated this newly available P in both roots and shoots.

Keywords KIT-6 · Mesoporous materials · Immobilized enzymes · Phytase · Environment · Phosphorus

1 Introduction

In agriculture, efficient plant growth most often requires fertilizing the soil with several mineral elements, among which phosphorus plays a crucial role in vegetal development. The manufacture of most fertilizers containing phosphorus relies on limited mine stocks. Because of the fast-growing worldwide demand for this resource, a phosphorus crash has been predicted and seems to be approaching [1]. This shortage in Phosphorus (P) resources, together with eutrophication issues due to P accumulation, calls for a better and more sustainable management of the P supply [2]. Organic P is partly stored in soils in the form of inositol phosphate (phytate), which could be an alternative source of P, provided the phytate can be hydrolyzed to produce the orthophosphate ions required for plant nutrition [3–5], and may help to reduce legacy soil P [2]. Phytase is an enzyme produced by many fungi and bacteria, which specifically hydrolyze phytate, thus releasing inorganic phosphate into the medium.

Plants have a very low capacity to release this phytase into their root environment [6, 7] and benefit from an association with the aforementioned microorganisms located in close vicinity to their root system. The main

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limitations of this association for optimal plant nutrition are the short-term viability of phytases released by microorganisms in the environment [4] and the very low diffusivity of inorganic phosphate in soils [3]. These limitations could be partly overcome by providing the plants with enzymes immobilized in a porous network with sustained activity located near the hair root system. Organic scaffolds, typically comprised of natural polymers (alginate, gelatin, chitosan, etc.), would be good candidates except for the usually large porosity of the hydrated gels [8] and their rapid biodegradation in soils due to soil enzymes (proteases, chitinases, cellulases) secreted by microorganisms. Alternatively, inorganic particulate materials have been used to encapsulate proteins. Such inorganic materials are resistant to environmental conditions and to enzymatic degradation. Clays were not considered because of the strong protein absorption and denaturation on such charged surfaces. Instead, we turned to siliceous materials originally developed for fine chemistry purposes, with well-defined nanometer-size porosity and pore dimensions that can be tuned to accommodate most proteins [9]. The encapsulated proteins can be protected from denaturing agents and even stabilized by their interaction with the pore surface, thus increasing their lifetime [10, 11]. Confinement and the local chemical environment inside the pores can even lead to increased activity of the immobilized enzymes [12]. For some applications, especially in the fine chemical industry, such supported catalysis allows for the easy recycling of expensive catalysts by simply filtering off the supporting particles.

In the present study, we were aiming to immobilize and stabilize phytase enzymes into porous silica microparticles to be used as alternative fertilizing agents, thanks to their ability to mobilize inorganic P naturally present in most agricultural soils. First, we synthesized and fully characterized KIT-6 mesoporous materials with a tri-dimensional porous structure and pores large enough to accommodate the rather bulky phytase molecule (ca. 11 × 8.5 × 8.5 nm^3). In parallel, MCM-41 silica with pores smaller than the shortest axis of the enzyme, was used here as a negative control for the enzyme intrusion inside the material. We report herein the adsorption behavior and kinetics of phytase onto and into both materials and assess the subsequent structural changes to the porosity of the supporting materials. We studied the enzymatic activity of the phytase-loaded materials in terms of the main enzymatic kinetic features (substrate concentration, temperature, pH) and, most importantly, the protection of phytase against protease activity. Finally, as a proof of concept we report on the enhanced accumulation of P in *Medicago truncatula* plants grown in the presence of phytase-loaded mesoporous materials with phytate as the sole exogenous source of P.

2 Materials and methods

2.1 Synthesis of mesoporous materials

The synthesis of large-pore silica materials such as KIT-6 was performed in solution under strongly acidic conditions ($pH < 1$) [9, 13]. The synthesis was performed by addition of 4.07 g of tribloc-copolymer pluronic P123 (Aldrich), 8 g HCl 36 % and 144 g H_2O. The mixture was stirred at 35 °C until complete dissolution of the pluronic P123, and 3.98 g of butanol (Prolabo) were added and stirred for another 2 h. Finally, 8.6 g of TEOS (Fluka) were added to the mixture and stirred for 24 h. Overall, the molar ratios for the synthesis of KIT-6 were 1 TEOS/0.017 P123/1.3 BuOH/5.308 HCl/194 H_2O. The resulting solid was filtered and dried at 80 °C.

MCM-41, a classical bi-dimensional mesoporous material [14], was synthesized with small pores (<4 nm) and used as a negative control for enzyme adsorption experiments. It was obtained by mixing 2.6 g $C_{18}TAB$ (Aldrich) and 4 g SiO_2 (Aerosil© 200, Degussa) in a basic solution ($pH > 13$) with 0.66 g NaOH (Prolabo) and 32.5 g H_2O. According to the procedure developed by Galarneau et al. [15], two hydrothermal post-treatments were performed to strengthen the pore walls and improve the stability of the material during calcination. This treatment is a dissolution/redeposition process that decreases the radius of curvature on the particle surface and at the pore entrance [15, 16]. The molar ratios for the synthesis mixture of MCM-41 were 1 SiO_2/0.1 $C_{18}TAB$/0.25 NaOH/54.5 H_2O. Both hybrid materials were calcined at 550 °C for 8 h in a muffle furnace, following a heating ramp at a rate of 2 °C/min.

2.2 Porous texture characterization by nitrogen volumetry

Nitrogen volumetry was used to determine the main textural characteristics of the materials (pore diameter, mesoporous surface area, porous volume), and their evolution upon exposition to solutions of the enzyme [17, 18]. Adsorption–desorption isotherms were obtained at 77 K with an ASAP2020 apparatus from Micromeritics. Prior to the sorption analysis, the samples were outgassed for 6 h (10^{-5} Torr) at 250 °C for the as-synthesized ordered silica, or at 100 °C for the enzyme-impregnated samples to avoid protein calcination. The samples surface area was determined by the BET (Brunauer, Emmett and Teller) method.

2.3 Enzyme properties

The phytase used in the present study (*myo*-inositol hexa*kis*phosphate phosphohydrolase, EC 3.1.3.8) belongs to the group of histidine acid phytases able to hydrolyze 5 of the 6 phosphomonoester bonds of phytate. We worked with a recombinant protein produced by overexpressing the Phy-A gene from *Aspergillus niger* in yeast (*Pichia pastoris*) using methanol as a promoter, according to Boze et al. [19] and Brunel et al. [20]. The phytase has a molecular mass of 85 kDa and an isoelectric point of 4.8 [21–23]. The enzyme extract, pure at 99 %, was produced at the "Halle de Biotechnologie" (UMR IATE/UMR SPO, Montpellier, France) with an initial phytase activity of 59.8 ± 2.3 U/mL of enzyme extract (with 1 U = 1 μmol of orthophosphate produced per minute). A total protein mass concentration of 45 mg/mL was determined in the extract by a BCA Protein Assay (Pierce), yielding a specific activity of approximately 1,330 U/mg of phytase.

2.4 Phytase activity measurements

The phytase activity was measured by quantifying the rate of release of orthophosphate ions (Pi) from ultrapure sodium phytate (Sigma-Aldrich, Ref P0109). Unless otherwise stated, the activity was measured using the diluted free enzyme (1/30,000) or a fixed quantity of functionalized mesoporous solid by the difference of Pi concentration at time zero t_0 and after 5 min of incubation with the substrate at 37 °C, t_5. The reaction mixture contained 300 μL of acetate buffer (0.5 M, pH 5.5), 100 μL of enzyme sample (solution or dispersed mesoporous particles), and 100 μL of phytate solution in the same buffer at the concentration indicated in the figure captions, typically 5 mM. The reaction was stopped with 500 μL trichloroacetic acid (1N) either before adding the substrate (time zero) or after incubation. The free Pi concentration was determined by adding 100 μL of CH_3COONa 1 M–100 μL of the acidic incubated sample to neutralize the sample before the addition of the Malachite Green's reactants. Afterwards, 40 μL of each of the two reactants used in the Malachite green method [24] were added before reading the absorption at 630 nm.

2.5 Effect of pH and temperature on phytase activity

The enzyme activity was studied over pH values ranging from 3.5 to 8. Buffer solutions of different pH were made by adding acetic acid to sodium acetate (0.5 M), and used to run the enzyme reaction as described above.

The temperature dependence of phytase activity was studied at pH 5.5 in the 5–75 °C temperature range with 10 °C increments. At each temperature, the substrate was pre-incubated for 5 min at the target temperature before addition of the enzyme. All measurements at different temperatures were made with independent samples. The temperature stability of the free and immobilized phytase was assessed at two extreme temperatures well below (4 °C) and above (55 °C) the expected denaturation temperature [25]. The samples were kept at a given temperature for 10, 20 and 30 min, and then rapidly cooled on ice for 10 min. The samples were equilibrated at 37 °C for 5 min before measuring the enzymatic activity, as described above.

2.6 Phytase loading, adsorption kinetics and adsorption isotherm

Based on the phytase dimensions and the porous volume determined for KIT-6, we estimated that approximately 450 μg of proteins loaded head-to-tail into the elongated pores would fill 100 μL of KIT-6 porous volume. In what follows, 1× thus corresponds to 450 μg of phytase for 100 μL of porous volume. Given the porosity characteristics of both materials, 100 μL of porous volume corresponds to 75.2 mg of KIT-6 and 130 mg of MCM-41. The effect of the adsorption behavior of the phytase was studied by varying the pH of the medium around the phytase isoelectric point (pI = 4.8). Given that the point of zero charge (PZC) for silica is approximately pH 2 [26], the mesoporous surface is negatively charged for all values of pH tested: 4, 4.8 and 5.5. The adsorption kinetic and adsorption isotherm experiments were performed under batch conditions at 18 °C. The mesoporous solid was initially prehydrated with 5 mL of buffer solution (pH 5.5) and kept under stirring for 30 s. For the adsorption kinetic, a mass of solid representing a porous volume of 100 μL as explained above was immersed in a solution containing 45 mg of phytase (100×) and kept under gentle stirring. At different times (0′/10′/20′/40′/1 h/3 h/24 h/48 h), the sample was centrifuged for 3 min and 250 μL were withdrawn from the supernatant for total protein titration. The adsorption isotherm was constructed by keeping the mesoporous silica in contact with increasing phytase concentrations under gentle stirring for 3 days; for KIT-6: 1×, 2×, 10×, 30×, 50×, 100×, 300× and for MCM-41: 1×, 10×, 30×, 50×, 100×. The proportion of enzyme immobilized into and onto the silica materials was estimated with the BCA assay by subtracting the amount of protein remaining in the supernatant from the total amount initially introduced for impregnation.

The Langmuir–Freundlich adsorption model [27] was used to describe the adsorption behavior of phytase onto mesoporous materials with the following general equation:

$$q_e = \frac{q_m K_{LF} C_e^n}{1 + K_{LF} C_e^n}$$

where, q_e is the amount of phytase loaded onto the mesoporous material at equilibrium (μg phytase/mg mesoporous material); q_m is the maximum adsorption capacity, it represents a measure of total number of binding sites available per gram of sorbant (µg phytase/mg mesoporous material); C_e is the supernatant concentration at equilibrium (µg/mL); K_{LF} is the affinity constant for adsorption in the langmuir–freundlich model (L/mg); n is the Langmuir–Freundlich coefficient, and was adjusted to $n = 0.95$ to best fit the experimental data.

2.7 Proteolytic treatment

KIT-6 impregnated with phytase was submitted to proteolytic treatment with trypsin to assess the protective effect of enzyme confinement into the mesopores. The standard amount of mesoporous solid impregnated with phytase (10×, 50×, 100×) was centrifuged, the supernatant discarded, and 200 µL of bovine trypsin solution (0.05 %) were added for 5–20 min at room temperature (25 °C). The solids were then thoroughly rinsed, and the remaining phytase activity was determined as described above.

2.8 Plant growth experiment

Medicago truncatula L. *Jemalong* strain A17 (from INRA, Mauguio, France) [28] was used as a plant model in our experiment. The seeds were sterilized by soaking them for 5 min in a solution of $CaClO_2$ (30 g/L) under agitation and washed with sterile deionized water. The seeds were kept wet at 4 °C for 4 days to break their dormancy before being placed to germinate in Petri dishes (12 × 12 cm) containing sterile agarose gel [10 g/L agarose (QBiogene, ref. AGAH0500) and 2 g/L glucose] for 3 days at 26 °C in the dark. Then, the plantlets were grown in Petri dishes (12 × 12 cm) filled with fine sand (50–70 particle mesh, Sigma-Aldrich, ref. 14808-60-7) washed repeatedly with deionized water and absolute ethanol and sterilized by drying overnight at 150 °C. First, the sand (99 g) was soaked with 16.5 mL of a MES (2-(N-morpholino)ethanesulfonic acid) buffer solution at pH 5.5. To avoid nutritional deficiencies, 8 mL of a mineral solution were added with the following composition: KNO_3 (0.1 M), $CaCl_2$ (0.07 M), FeEDDHA (140 µM) and micronutrient solution (2 mL/L). The micronutrient solution was composed of H_3BO_3 (45 mM); $(NH_4)_6MoO_7O_{24}$ (0.3 mM); $ZnSO_4$ (15 mM); $MnSO_4$ (18 mM); $CuSO_4$ (4 mM), supplemented with 1.6 mL of $MgSO_4$ (1 M). A final concentration of 2.7 mM Na-phytate (Sigma ref. P0109) was reached by adding 5 mL of a concentrated solution sterilized by filtration at 0.2 µm were then added and considered as the sole source of phosphate. In one set of conditions, the nutrition solution was devoid of phytate, and instead KH_2PO_4 was added (final concentration of 49 mM), thus providing a non-limiting source of free inorganic phosphorus from the start of the experiment. Finally, except for negative control conditions in the absence of enzyme, the phytase-loaded mesoporous solid KIT-6/10× was added as a suspension in water (1 mL at 7.52 g/L). Ultrapure water (18 Mohm) adjusted to pH 5.5 with HCl (12N) was used to complete the soil solution volume to 33 ml. The plants (four per dish) were placed in sterile conditions and grown for 30 days at approximately 26 °C with a photoperiod of 11 h of natural light supplemented with mercury vapor lamps during the night and watered with ultrapure water at pH 5.5 regularly to compensate for evaporation. Afterwards, the plants were harvested and the soil solution was brought back to its initial volume by addition of ultrapure water before measuring the free inorganic P concentration. Each plant was analyzed separately, the shoots and roots being separated for acid mineralization with 10 mL HCl (6N) at 110 °C for 16 h [29]. The Malachite green method was used to determine the P quantity in the soil solution and in the mineralized plants.

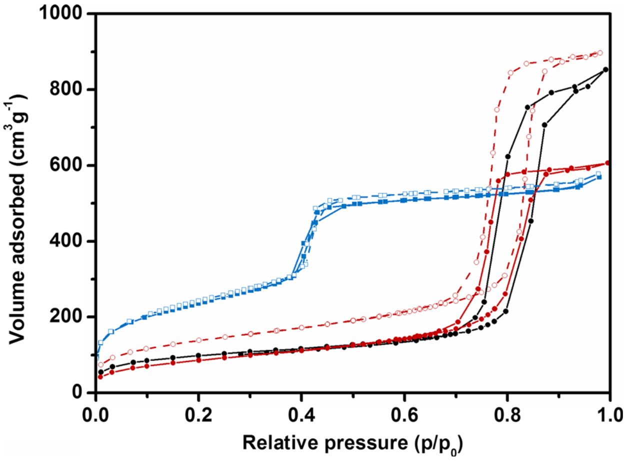

Fig. 1 Nitrogen absorption/desorption isotherms at 77 K for as-synthesized KIT-6 (*red open circles*) after impregnation KIT-6/I (*red closed circles*) and after re-calcination (550 °C for 8 h) KIT-6/IC (*black closed circles*); for as-synthesized MCM-41 (*blue open squares*) and after impregnation MCM-41/I (*blue closed squares*). All samples have been impregnated with 45 mg phytase (100×) for 3 days (Color figure online)

3 Results and discussion

3.1 Protein immobilization into mesoporous materials

The porosity of the calcined materials, before and after protein adsorption, was characterized using nitrogen sorption. All materials, before and after phytase immobilization, gave type IV isotherms with an H2-type hysteresis loop (Fig. 1) typical of mesoporous materials with moderate pore size distribution [17]. The main porous features of the materials, "as synthesized" and after enzyme impregnation, determined by nitrogen sorption, are summarized in Table 1.

Table 1 Main porous features of the materials as determined by nitrogen adsorption/desorption volumetry: pore diameter, pore volume and BET surface area

Mesoporous silica	Pore[a] diameter (nm)	S_{BET} (m^2g^{-1})	Pore[a] volume (cm^3g^{-1})
MCM-41	3.8	875	0.8
MCM-41/I			
1×	4.5	854	1.0
50×	4.3	682	0.6
100×	3.8	625	0.5
KIT-6	8.6	480	1.4
KIT-6/I			
1×	8.9	476	1.5
30×	8.4	344	1.0
100×	8.2	311	0.9
KIT-6/IC	9.5	330	1.3

[a] Calculated from desorption branch by BJH method

Under our conditions, we found a pore diameter of 3.8 nm for MCM-41 and 8.6 nm for KIT-6 for the as-synthesized materials, in good agreement with previous studies [9, 13, 14]. As expected, the longest axis of the *A. niger* phytase (PDB entry 3K4P [30]) being slightly below 7.5 nm, the protein is bigger than the pores of MCM-41 but smaller than the pores of KIT-6. Upon loading with a large excess of enzyme (100×) followed by washing and drying, both the porous volume and the specific surface area were decreased significantly by approximately 30 %, most likely indicating that part of the mesoporosity became inaccessible (Table 1).

The main changes in the N_2 sorption isotherm after re-calcination of impregnated KIT-6 are that the higher pressure plateau is not reached, and the hysteresis loop is less steep in the p/p° region (0.7–0.85) where condensation occurs in the mesopores. Such a shape for the hysteresis loop, which mostly reveals a less ordered interconnected porosity, is typical of a cage-like structure [31, 32] most likely originating from pore-blocking by the adsorbed protein. Partial dissolution and re-precipitation of silica may also occur upon impregnation with an aqueous solvent [15, 33], although such rearrangement of the silica network must be limited because the initial porous structure is mostly restored by calcination of the protein. The average Derjaguin-Broekhoff-de Boer (DBdB) estimated pore diameter was mostly unaffected by impregnation and re-calcination.

3.2 Phytase adsorption and desorption

We quantified the specific loading capacity of MCM-41 and KIT-6 by impregnating the materials with increasingly concentrated phytase solutions. Protein adsorption isotherms presented in Fig. 2a were in relatively good agreement with a simple Langmuir–Freundlich adsorption model in the lower concentration range. However, beyond a free phytase concentration of 3 mg/mL, the experimental data strongly deviated in excess of the values predicted by the model, which can be attributed to the formation of enzyme multi-layers adsorbed on the external surface.

The specific loading capacity expressed in microgram of phytase per milligram of material is approximately twice as large for KIT-6 compared to MCM-41, giving 6.3 and 3.5 µg/mg, respectively. We also observed that decreasing the pH of the impregnation solution to the enzyme isoelectric point, pI (pH 4.8) and below (pH 4) resulted in, respectively, an 8 and 3 % decrease in adsorbed amount with KIT-6, compared to pH 5.5. All experiments were performed well above the point of zero charge of silica ($pH < 3$) [26, 34], so the material surface is expected to be slightly negatively charged. A moderately repulsive electrostatic interaction with the negatively charged enzyme ($pH > pI$) thus appeared to improve adsorption, and all subsequent adsorption experiments were carried out at pH 5.5.

For both materials, the impregnation kinetics showed a relatively fast adsorption rate in the first 10 h of contact, with approximately 87 % of the protein incorporated, with respect to the amount measured at the quasi-plateau reached after 48 h (Fig. 2). In contrast, desorption was rather slow and amounted to approximately 0.05 % at the plateau reached after approximately 2 days. Indeed, the adsorption isotherm in Fig. 3a shows that for the condition 1×, approximately 3.2 µg of protein are adsorbed into KIT-6, which, given the specific activity of the free enzyme (1,330 U/mg), amounts to approximately 190 U/mg of material. Under the same conditions, only 0.1 U is released per mg of material (Fig. 2). Replacing the release solution by fresh buffer after 10 days did not result in more desorption, indicating that the release plateau was not due to saturation of the release medium (Fig. 2).

3.3 Immobilized enzymatic activity

For KIT-6, the incorporated phytase activity was approximately 8 U per mg of mesoporous material with an initial loading amount of 4.5 mg phytase (Fig. 3b), suggesting that a large proportion of the enzymatic activity is limited by the diffusion of the substrate through the mesopores. The relatively low apparent enzymatic activity with respect to the amounts of protein effectively loaded, 9 U instead of 33 U (Fig. 3a), i.e., ∼30 %, is therefore likely due mostly to enzymes located at the surface of the particles [35].

Phytase activity measured as a function of phytase concentration in the incubation medium followed relatively

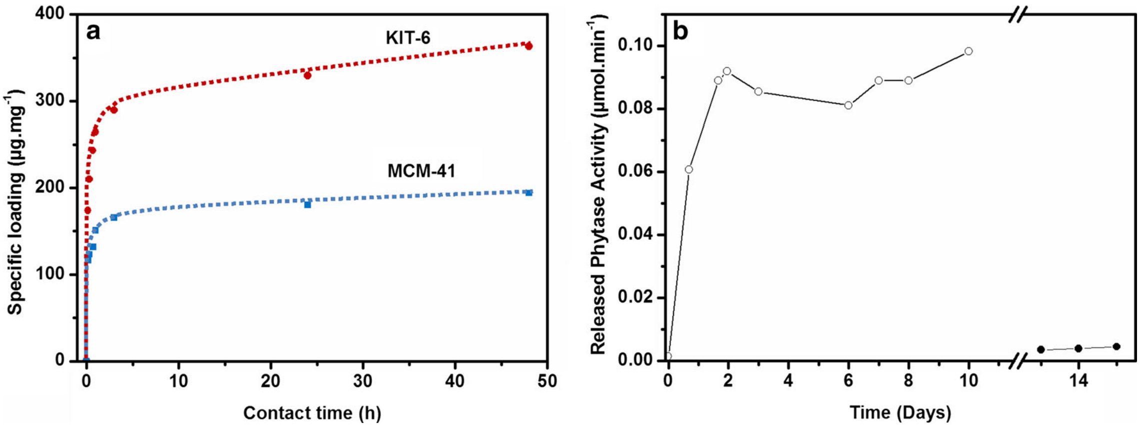

Fig. 2 Adsorption and desorption kinetics of phytase on mesoporous silica KIT-6 (*red circles*) and MCM-41 (*blue squares*). **a** Adsorption kinetics measured on mesoporous materials loaded with a quantity equivalent to 100× (see "Materials and methods" section). **b** Activity of the phytase desorbed from a previously impregnated KIT-6/I (1×), in 10 mL of acetate buffer at pH 5.5. Phytase activities were measured at pH 5.5, 37 °C and with a phytate concentration of 5 mM (Color figure online)

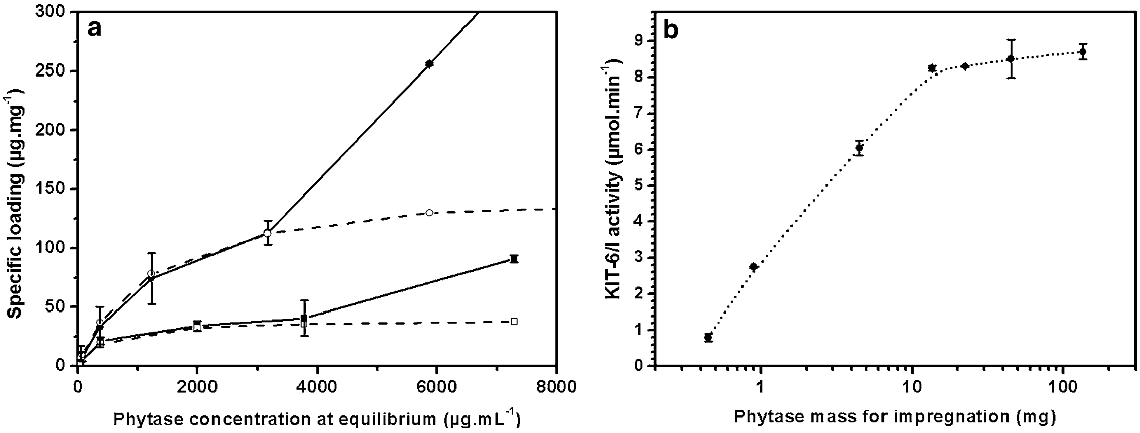

Fig. 3 **a** Phytase adsorption isotherms for KIT-6/I and MCM-41/I. The materials were impregnated for 3 days by immersion in enzyme solution at different concentrations. Calculated isotherms, according to the Langmuir–Freundlich model (see Ref. [24]), are drawn in dotted lines (n = 0.95). **b** Phytase enzymatic activity measured in KIT-6 materials as a function of the amount of phytase used for loading. The temperature was 37 °C, and the phytate concentration was 5 mM

similar evolutions for the free and for immobilized phytase with a typical hyperbolic curve (Fig. 4a). As expected, the enzyme activity was much stronger for the free phytase than for the immobilized phytase, owing to the diffusion constraints in the latter case. A slight decrease in activity was noticeable at the highest substrate concentration under all conditions, apparently less pronounced for the enzyme associated with KIT-6 (12 %) than for the free enzyme or associated with MCM-41 (20 %). The maximum activity obtained for KIT-6 impregnated with the enzyme was approximately twice the maximum activity reached with MCM-41 under the same conditions.

For the freshly immobilized enzymes only, a striking singularity was visible in the low substrate concentration region, below 40 µM (Fig. 4b). In both cases (KIT-6 and MCM-41), the activity went through a first maximum at approximately 10 µM phytate and then decreased slightly to a secondary minimum at 15 µM phytate before increasing again. The phytase activity then reached a plateau in the 20–40 µM region and increased once again to finally reach V_{max} at approximately 200 µM phytate (Fig. 4a). However, upon aging for 4 months at 4 °C in acetate buffer at pH 5.5, both materials appeared to evolve, yielding much smoother curves with a slightly lower activity in this low concentration range. We attribute the succession of concentration regimes with the impregnated mesoporous materials to distinct locations of the enzymatic activities, with a different accessibility to the substrate. In particular, it is likely that, in the case of MCM-41, because the protein is too large to enter the porosity, most of the protein is adsorbed on the outer surface of the particles with direct access to the substrate at the bulk concentration.

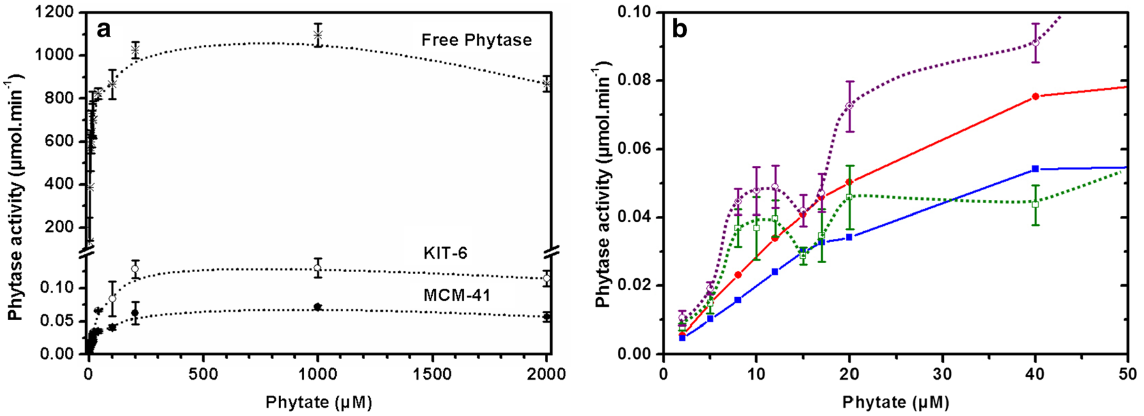

Fig. 4 Phytase activity of the free phytase (*stars*), KIT-6/I (1×) (*circles*) and MCM-41/I (1×) (*squares*) freshly impregnated as a function of substrate concentration. **a** Phytase activities measured over the whole range of phytate concentrations studied (0–2 mM). **b** Zoom of (**a**) in the low phytate concentration region for freshly impregnated materials (*open symbols*, *dotted line*) and the same samples after 4 months aging (*full symbols*, *straight line*). The *lines* are guides to the eye (Color figure online)

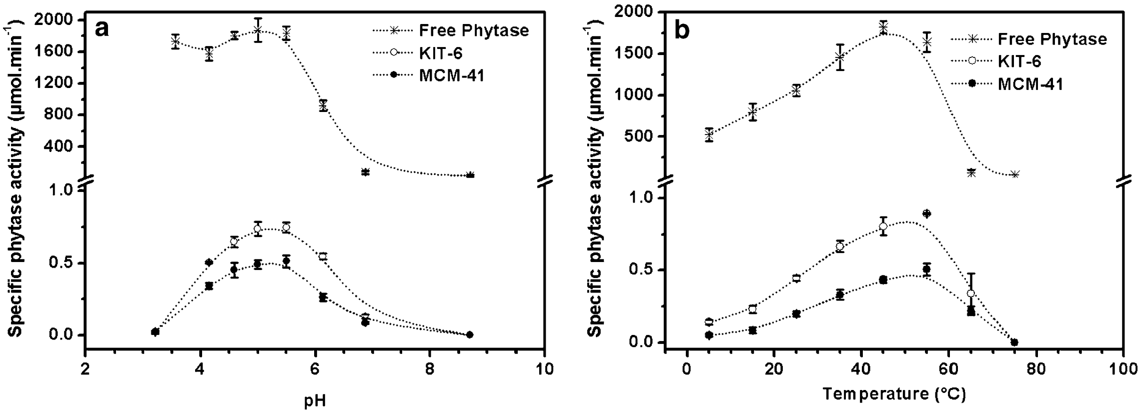

Fig. 5 Effect of pH and temperature on phytase activity whether free in the solution or freshly impregnated in the mesoporous silica KIT-6/I (1×) and MCM-41/I (1×). **a** The effect of the pH on activity was measured at 37 °C with 2 mM phytate. **b** The effect of the temperature on activity was measured at pH 5.5 with 2 mM phytate

The diffusion constraints are those that apply to catalytic species attached to a flat surface [35]. Another smaller proportion of the enzyme can be positioned at the mouth of the pores with less accessibility to the substrate. This second population would also account for the partial pore blocking observed for both materials by nitrogen adsorption upon impregnation (Table 1). In the case of KIT-6, a third population of enzymes can be inserted into the internal porosity with even more limited access to the phytate due to the diffusion constraints and the upstream enzymatic activities causing substrate depletion inside the pores. After 4 months of aging, the desorption and denaturation of the enzymes adsorbed on the surface, together with the rearrangement of the silica network in aqueous buffer, could lead to a more homogeneous repartition of the catalytic sites (Fig. 4b) and explain the smoother aspect of the curve compared to fresh samples.

For both impregnated mesoporous materials and the free enzyme in solution, the optimal pH was located in pH values ranging from 5 to 5.5 (Fig. 5a). The activity of the functionalized materials became very limited at the lower (pH 3.2) and upper (pH 8.7) ends of the pH range investigated, in contrast to the free phytase behavior that also showed a strong decrease in activity at higher pH but had a second optimum of activity at acidic pH, which was reported to be located at approximately pH 2.5 [23].

Similarly, the optimum temperature lies in a 45–55 °C region for the immobilized and free phytase. However, as Fig. 5b shows, the immobilized enzyme activity increases in this range while the immobilized enzyme activity of the free phytase starts decreasing. The optimum temperature T_{max} therefore appears to be slightly larger for the immobilized than for the free enzyme.

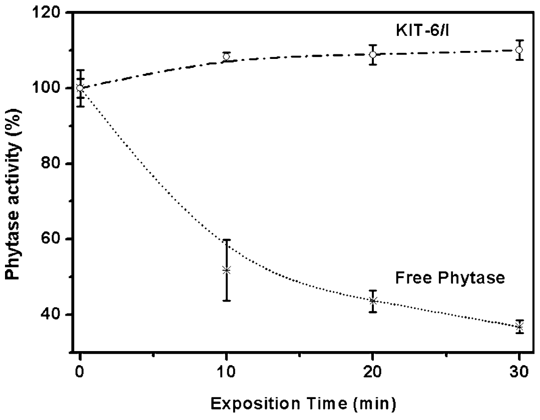

Fig. 6 The temperature stability for the impregnated mesoporous silica KIT-6/I (**1×**) and the free phytase. The samples were exposed at 55 °C for the desired duration and then rapidly cooled to 4 °C. Activity measurements were performed after equilibration at 37 °C (pH 5.5), with a phytate concentration of 1 mM

To evaluate the possible stabilizing effect of immobilization into the mesoporous material, the evolution of the enzyme activity was followed at 55 °C, which corresponds to T_{max} for the immobilized phytase. It clearly appeared (Fig. 6) that under these conditions the free enzyme was not stable, as its activity dropped rapidly with time at 55 °C (down to 20 % after 48 h, data not shown), while the activity of the immobilized phytase remained mostly constant over 30 min.

3.4 Protection of immobilized phytase against protease activity

The set of data presented so far strongly suggested that at least part of the phytase used to impregnate KIT-6 mesoporous silica is immobilized inside the porous network, possibly accounting for higher temperature stability compared with the free enzyme. However, phytase adsorption isotherms (Fig. 3a) suggested that for high protein amounts, multilayers may form on the outer particles surface. It is difficult to determine directly the proportion of enzyme actually included in the porosity based on the overall enzymatic activity because of the very different substrate diffusion constraints between the particle surface and sites located deep in the pores. We used bovine trypsin, a small protease (4 nm in diameter), to remove the protein adsorbed in excess outside the porosity. The enzymatic activity associated with the porous materials was evaluated before and after trypsin treatment. The initial rate obtained for KIT-6/I after trypsinization was about one-third of the rate of the initial sample (Fig. 7a), indicating clearly that part of the enzyme associated with the material had been hydrolyzed. Although trypsin is much smaller than the pore diameter, preliminary tests showed that the proportion of removed phytase did not evolve much with time up to 20 min (Fig. 7b), strongly suggesting that the adsorbed phytase is concealed deep within the pores.

Interestingly, the trypsinization of samples impregnated 10×, 50× and 100×, i.e., with an increasing excess of protein, gave an identical remaining phytase activity (Fig. 7a). In the case of KIT-6/10×, this amounts to nearly 35 % of the initial activity measured before treatment. For the sake of comparison, it can be noted that MCM-41/1× conserved only 7 % of its apparent enzymatic activity after the same treatment. This relatively large remaining phytase activity for KIT-6 after trypsin treatment is in good agreement with our hypothesis of enzymes filling the accessible porosity and the excess proteins forming multiple layers on the outer particles surface. The remaining activity was approximately 2.7 phytase units, whatever the initial amount used for impregnation. This remaining

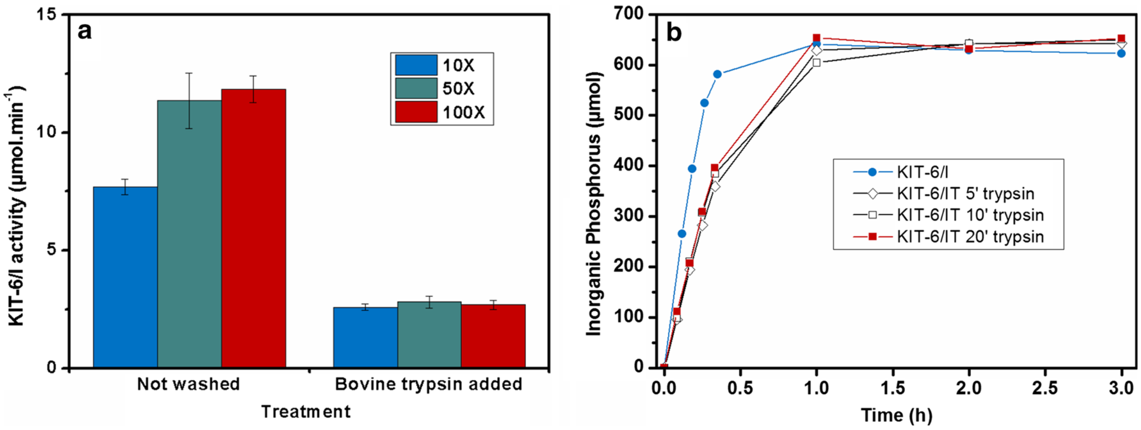

Fig. 7 Effect of trypsin treatment on the activity of phytase-loaded KIT-6. **a** Enzymatic activity of samples loaded with increasing amounts of phytase (10×, 50× and 100×), before and after a 5 min treatment with trypsin. **b** Evolution of enzymatic activity kinetics for KIT-6/I (1×) before and after (KIT-6/IT) exposition to trypsin (500 µL at a 0.5 g/L concentration) for 5, 10 and 20 min. Measurements were done at 37 °C and pH 5.5 with 5 mM phytate (Color figure online)

Table 2 Analytical results of plant growth experiment with (+ plants) or without (− plants) and with (+ KIT-6/10×) or without (− KIT-6/10×) phytate-impregnated mesoporous material

	Treatments	Amount of free Pi in solution (µmol/Petri dish)		Dry mass (mg/plant) in		Total P accumulation (µmol/plant)
		t_{initial}	t_{final}	Roots	Leaves	
+ plants	+ phytate − KIT-6/10× (control 1)	0.3 (0.3)	4.7 (2.1)	4.5 (3.6)	32.6 (11.0)	4.2 (0.8)
	+ phytate + KIT-6/10×	15.7 (3.3)	154.1 (30.6)	7.4 (1.2)	23.9 (19.3)	10.8 (9.4)*
	+ KH_2PO_4 (49,25 mM) (control 2)	64.3 (0.7)	28.9 (8.2)	4.3 (2.6)	25.5 (5.5)	26.2 (8.7)*
− plants	+ phytate − KIT-6/10× (control 1)	0.18 (0.13)	0.68 (0.08)			
	+ phytate + KIT-6/10×	301.6 (115.3)	218.79 (28.3)			
	+ KH_2PO_4 (49,25 mM) (control 2)	65.0 (5.2)	57.6 (1.9)			

Data are means with standard deviation between brackets (n = 6) and correspond to (1) the amount of free orthophosphate (Pi) assayed in solution before (t_{initial}) and after harvest (t_{final}), (2) the dry biomass (mg) of leaves and roots and (3) the total P accumulated in *Medicago truncatula* plants. Total P accumulated was obtained by addition of P content of leaves and roots. P content was obtained by multiplying dry biomass per % P (data not shown)

* t-student comparison of the mean with mean of control 1, significant at $p < 0.05$

activity corresponds to 20 % of the activity expected at 1× for the free enzyme. Such a discrepancy most likely arises from only partial filling of the pores but also from the limited diffusion of the substrate inside the porosity, which lowers the apparent activity of the immobilized enzyme.

3.5 Phosphorus available for growing plants

We then investigated whether the inorganic P released by the hydrolysis of phytate catalyzed by the KIT-6-bound phytase was efficiently taken up by growing plants. We chose *M. truncatula* as a model plant because of its rapid growth and its low seed P content, making the plantlets highly dependent upon external P for their growth. The main data regarding the free Pi available in the soil solution, dry mass of plantlets after 30 days cultivation and total P accumulation are summarized in Table 2. Overall, all the conditions tested proved suitable for plant growth and mostly differed in both available free Pi and plant accumulation of P.

First, the amount of inorganic phosphorus (Pi) available per dish was evaluated after 30 days incubation with or without KIT-6/10×, in the absence or presence of plants (Table 2). Obviously, the amount of Pi for the negative control (no phytase, no plant) is extremely low and most likely corresponds to spontaneous hydrolysis of phytate or contamination by inorganic phosphorus in the phytate solution. As expected, in the presence of phytase-loaded KIT-6, the amount of Pi available at the beginning of the experiment was largely increased to approximately 300 µmol Pi per dish. However, after 30 days in the absence of plants, the concentration of free Pi decreased to approximately 220 µmol Pi per dish (Table 2), likely due to spatial variability and low mobility of P and phytate. The presence of plantlets (four per dish) for 30 days resulted in a net decrease of approximately 60 µmol of Pi per dish. Even in the absence of phytase-loaded KIT-6, the presence of the plant induced an increase in the Pi concentration. This increase is most likely due to the production by *M. truncatula* of its own phytase whose secretion was hypothesized by Xiao et al. [36]. However, this enzyme is able to hydrolyze phytate only at a limited yet significant ($p = 0.0004$) extent. Finally, to assess the ability of the plants to absorb the Pi released by the hydrolysis of phytate catalyzed by the enzyme loaded-material, the P content in

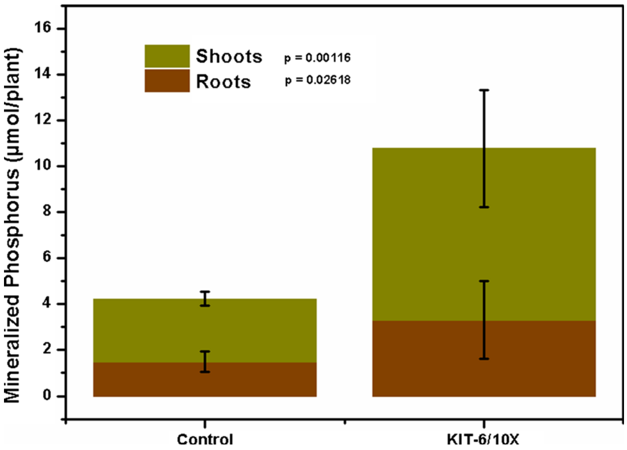

Fig. 8 Total amounts of P assayed in roots (*brown bars*) and shoots (*green bars*) in *M. truncatula* seedlings grown for 30 days under sterile conditions with phytate as the sole source of P, without (Control) or with (KIT-6/10×) the phytase-containing mesoporous material. Bars are the means with SEM (n = 5). The results of the F-test are given, showing that P amounts accumulated in roots and shoots of plants grown with KIT-6/10× were statistically greater than the P amounts measured in plants grown without KIT-6/10× (Color figure online)

the roots and shoots of each plant was determined (Fig. 8). The amount of P accumulated by the plant in the presence of KIT-6/10× is approximately 10 µmol per plant, 2.5 times higher than the control, and the difference is significant both in roots and shoots. This accumulation in the plants accounts for most (66 %) of the net decrease in Pi concentration in the soil solution between the conditions in the absence and presence of the plant. It should be noted that when inorganic P is added directly to the nutrition medium at a non-limiting concentration (KH_2PO_4 49 mM), the total P accumulated by the plants amounts to 26.2 ± 8.7 µmol/plant, which is significantly higher ($p = 0.0187$) than with 2.7 mM phytate and KIT-6 immobilized phytase (Table 2).

4 Conclusions

Immobilization of a phytase capable of hydrolyzing phytate, an organic form of phosphate present in natural soils, was successfully achieved in KIT-6 porous silica. The pores of the 3-D cubic mesoporous network are indeed large enough (>8.5 nm in diameter) to accommodate the bulky phytase protein. Most of the adsorption into the porous network of the silica particles occurred within the first 10 h of contact. Very little was released back to the medium, indicating limited leaking and thus stable immobilization by simple adsorption. In the perspective of applications for supported catalysis, it is crucial that the enzyme stay associated to the supporting scaffold, allowing for the efficient recycling of the catalyst material. Although the kinetics might apparently be affected by adsorption onto the pore walls and by diffusion constraints on the substrate and products in the mesopores, the main enzymatic features remain very similar to those of the free phytase. Most interestingly for their use in soils as fertilizers, the enzymes loaded into KIT-6 appeared partially protected from protease degradation. In addition, as suggested by a slight shift in optimal temperature, the thermal stability of phytase was greatly enhanced upon immobilization in the mesoporous silica. Such stabilization could prove very useful for the processing of phytase with other components of the fertilizer compound, to improve shelf-life, and also for the long-term activity of the enzyme in soils. Finally, as a proof of concept, we demonstrated that not only is phytase-loaded KIT-6 efficient at hydrolyzing phytate but phytase-loaded KIT-6 also provides inorganic P that can be taken up by growing plants and accumulated in both roots and shoots. Our strategy consisting of mobilizing organic P accumulated in the soil (legacy P) is one way of addressing recent concerns regarding the management of P resources [2].

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Relevance of use-invariant soil properties to assess soil quality of vulnerable ecosystems: The case of Mediterranean vineyards

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ABSTRACT

Vineyard landscapes in Mediterranean areas represent a strong cultural legacy and support a crucial socio-economic sector. The sustainability of these landscapes is threatened by rapid changes of soil quality, in the context of global change and intensive management practices. Considering the Languedoc-Roussillon region of southern France as representative of Mediterranean soil and climate conditions, we evaluated the topsoil quality of vineyards at the regional scale, based on a set of 31 physical, chemical and biological soil indicators measured on 164 commercial vineyard plots. Almost all soil parameters were highly variable among plots. Biological activity was low but no systematic perturbation of the trophic web was noticed. The regional variability of use-invariant soil properties (such as calcium carbonate content and texture) was characterized and taken into account for the analysis of dynamic indicators. Invariant soil properties explained up to 25% of the variance of dynamic chemical and biological indicators. Consequently as a tool to improve soil management decision and recommendations, we proposed a grouping of the 164 vineyard plots into functional soil groups determined by soil invariant properties. Information redundancy between different dynamic indicators was analyzed, and their interpretation and limitations as indicators of topsoil quality were discussed. Our study has produced detailed topsoil indicator baselines that can be immediately used as references for winegrowers to appraise the topsoil quality of their vineyard in comparison with others.

1. Introduction

Vineyards deliver environmental, social and economic services to humankind. Since the classical or probably even the Neolithic era, grape vines have been cultivated in Europe. Landscapes of outstanding beauty such as Alto Douro, Lavaux or Saint-Emilion are world heritage sites listed by UNESCO and should be considered as a legacy of past generations. All over the world where grape vines are grown, they are associated with the concept of *terroir*. The *terroir* is defined as a unique ecosystem in a given place including soil, climate and the vine (Van Leeuwen et al., 2004). The *terroir* concept also embraces wine-growing and wine-making practices (Van Leeuwen and Seguin, 2006). Grape vines are grown in patches across the Mediterranean biome, in geographically distinct regions around the world: Europe (France, Portugal, Spain, Italy, Greece), North America (California) and South America, but also Australia and South Africa (Jones et al., 2005) and are often located on soils unsuitable for other crops. Winegrowers commonly practice chemical weeding, tillage, green pruning and intensive pesticide application in order to control yields and to obtain high quality grapes. There are side effects to these practices that can deteriorate soil functioning as they promote soil erosion, soil organic matter depletion, pollution and losses of biodiversity (Coulouma et al., 2006; Hildebrandt et al., 2008; Komarek et al., 2010; Lagacherie et al. (2006) Martinez-Casasnovas et al., 2009; Michaud et al., 2007; Nascimbene et al., 2012; Raclot et al., 2009). Vineyard soil functioning and associated ecosystem services are therefore threatened, especially in vulnerable areas such as

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Mediterranean climatic regions (Schroter et al., 2005), yet they are still poorly documented. Another factor affecting vineyard soils is the recent boom of conversions to organic management practices, but studies on the consequences of such changes on soil functioning are scarce and often assigned to a limited geographical area (Coll et al., 2011, 2012a). A comprehensive understanding of vineyard soil functioning in different *terroir* contexts and under the effect of changes in climate and viticulture practices is needed, and can be approached through the assessment of soil quality.

Soil quality is defined as the ability of soil to function as a vital living system and to deliver ecosystem services related to plant and animal production, regulation of nutrient cycles and pollution and conservation of biodiversity (Doran and Parkin, 1994; Doran and Zeiss, 2000; Karlen et al., 1997; Kibblewhite et al., 2008). Soil quality is evaluated for a specific land use and final user (Arshad and Martin, 2002). In practice, two different types of soil quality are distinguished (Karlen et al., 1997; Wienhold et al., 2004): (i) inherent (or use-invariant) soil quality and (ii) dynamic soil quality. The former is intimately linked to pedogenetic processes; it is almost invariant at the human scale and can be used for land-use planning. In contrast, dynamic soil quality is influenced by land use, agricultural practices, soil management and climate change and is consequently likely to vary over a short time period, especially for the topsoil, i.e. the upper soil layer (0–15 to 0–30 cm). These two types of soil quality are based on specific measurable parameters. Inherent soil quality parameters mainly include stoniness, particle size, soil depth and calcium carbonate contents. For dynamic soil quality stakeholders typically use chemical or physical parameters to evaluate total or available nutrient or water contents, levels of pollutants and risk of erosion. However, biological parameters have recently emerged as indicators able to provide a broader picture of soil quality due to their better sensitivity to environmental changes (Bastida et al., 2008; Fränzle, 2006). Potential biological indicators are numerous. Among them, microorganisms (mainly bacteria and fungi) and nematodes are particularly relevant for large-scale soil quality studies (Schloter et al., 2003) and are known to be particularly active in the topsoil. Soil nematodes are present in all soil types, occupying all trophic levels, and they are directly involved in the cycles of nutrients such as N and P (Irshad et al., 2011). In addition, indices based on the relative abundance of nematode functional guilds (combination of trophic group and life strategy) have been related to farming practices or land use (Bongers, 1990; Ferris et al., 2001; Villenave et al., 2001; Zhang et al., 2012). Microbial biomass, soil respiration, metabolic quotient, and the ratio of organic carbon from microbial biomass to total soil organic carbon are commonly recognized as indicators of the effects of agricultural practices or land use on soil quality (Bastida et al., 2008; Kaschuk et al., 2011; Mader et al., 2002; Probst et al., 2008).

The measurement and analysis of a spectrum of physical, chemical and biological indicators can thus provide a much-needed holistic understanding of soil quality in the particular context of Mediterranean vineyards. The appropriate scale for such a study is the regional scale, at which the wine-growing socio-economic sector is structured and which encompasses the field of action of a number of producer, consultant and commercial organizations. A regional scale evaluation provides a general assessment of soil quality for the whole region, while it also offers a framework allowing finer analyses for specific situations. This issue is particularly important for winegrowers who require management advice tailored to their *terroir* specificities. Contextualized soil management recommendations have as yet been made difficult by the inexistence of a complete and representative set of reference values for dynamic soil properties. The range of variation of dynamic soil properties can be partly determined by inherent soil properties (Dequiedt et al., 2009; Rutgers et al., 2008), and if this correlation is characterized and quantified it can help to produce easily consulted tables of reference values of dynamic properties for different soil types.

To attain these objectives we established an extensive network of 164 commercial Mediterranean vineyards in Southern France, covering a diversity of soils and management practices, on which we measured a large number of inherent and dynamic topsoil parameters. We deliberately promoted biological indicators in our study as (i) these indicators are not frequently used by wine growers in spite of their ability to provide important information on soil functioning and because (ii) physical indicators related to erosion processes (aggregate stability) or soil compaction have already been widely studied in Languedoc Roussillon vineyards and reference values are already available (see publications by Le Bissonnais et al., 2007; Raclot et al., 2009 or Coulouma et al., 2006 and Lagacherie et al., 2006).

We specifically aimed to: (i) produce an evaluation of the soil quality of Mediterranean vineyards through the analysis of a number of inherent and dynamic topsoil parameters, with emphasis on rarely studied biological parameters, and to (ii) quantify the contribution of inherent soil properties to dynamic soil quality in order finally to (iii) propose an operational classification of soils allowing the appraisal by winegrowers of their soil quality in a range of specific *terroir* contexts.

2. Materials and methods

2.1. Study sites and sampling procedures

Nine areas representative of the main wine-growing landscapes found in the Languedoc-Roussillon region were defined, as equivalents of the ecological zones proposed by Arshad and Martin (2002) to be relevant units for soil quality monitoring. The areas were delimited based on expert knowledge of regional geology, geomorphology and vegetation cover (Fig. 1). Within each representative area, plots were selected to cover the diversity of vineyard practices and included both organic and conventional vineyards. Altogether, 164 commercial vineyard plots were sampled as follows in the different representative areas: 18 plots in Aigues-Mortes (Ai-Mortes); 21 plots in Faugères (Faug); 19 plots in Jonquières-Saint-Vincent (J-St-V); 19 plots in Lesquerde (Lesq), 21 plots in Montagnac (Mont); 15 plots in Saint-Hippolyte-du-Fort (St-H-du-F); 23 plots in Saint-Victor-la-Coste (St-V-la-C); 11 plots in Terrats (Terrats) and 17 plots in Vergèze (Verg). The surface area of the studied plots ranged from 0.2 to 2 ha.

The climate in the Languedoc-Roussillon region is typically Mediterranean, with a mean annual temperature of 14.7 ± 0.6 °C and a mean annual rainfall of 694 ± 58 mm (averages based on data collected from 2000 to 2010 by Météo-France in nine weather stations representative of the studied areas). Soils were sampled between March 5 and May 22, 2009, with no more than 2 consecutive days of sampling for each area. For each of the 164 plots, ten topsoil sub-samples were taken randomly in the vineyard from 0 to 15 cm layer at the center of the inter-row, using a gouge auger. Sub-samples were carefully homogenized to form a single representative composite topsoil sample per plot.

2.2. Soil analyses

Thirty-one parameters were analyzed: 7 inherent physical parameters, 8 dynamic chemical parameters and 16 dynamic biological parameters (Table 1). We decided to include pH in dynamic parameters because soil pH evolves during the transformation and cycling of carbon and nitrogen, both influenced by vineyard management (Bolan and Hedley, 2003), and because liming practices can result in significant changes in soil pH.

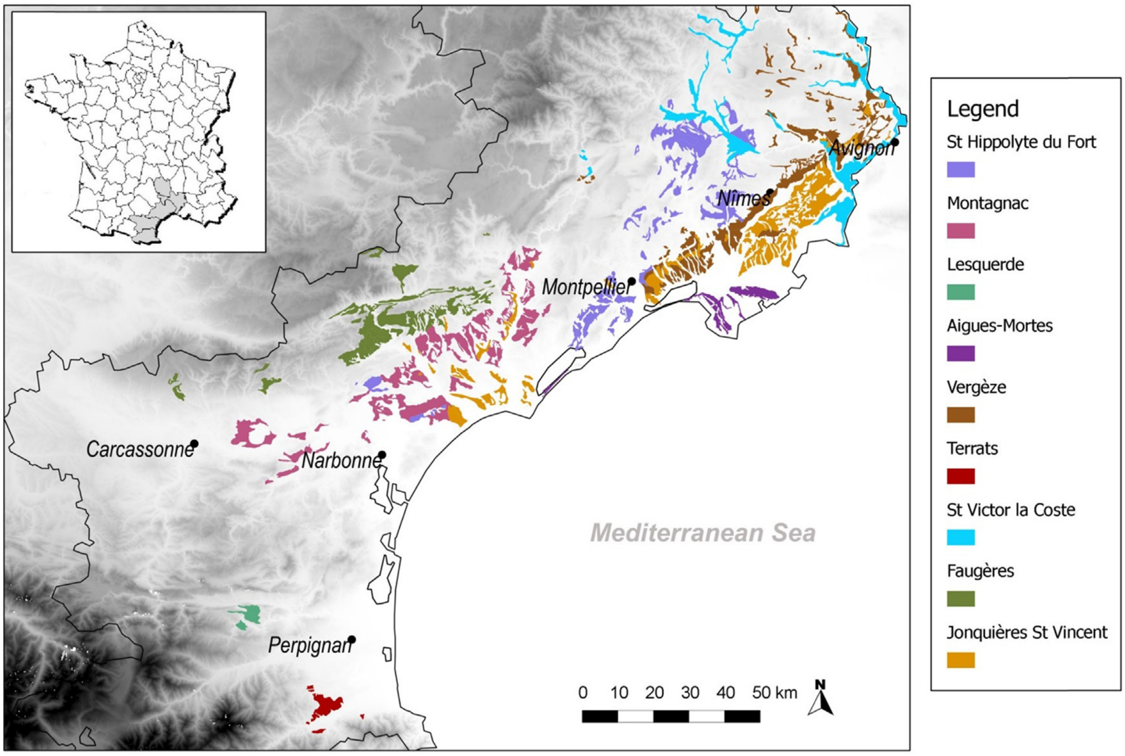

Fig. 1. Localization of the nine representative areas where the 164 vineyard plots were sampled. Each zone was given the name of the town where the majority of plots were sampled. The top left box situates the Languedoc-Roussillon region (in gray) on an administrative map of France. Map background: SRTM Digital Elevation Model, 90 m resolution.

The proportions (w/w) of soil particles larger than 1 cm in diameter (P_1 cm) and between 0.2 and 1 cm (P_2 mm) were determined by sieving and weighing. Soil texture, total and active calcium carbonate ($CaCO_{3\,tot}$ and $CaCO_{3\,act}$), organic carbon (C_{org}) and total nitrogen (N_{tot}) contents and the effective cation exchange capacity (CEC), as determined with the Cobaltihexamine method, were measured at the routine, national Laboratory of Soil Analyses of INRA (LAS INRA-Arras, France), using standard methods on 2 mm-sieved soil (Pansu and Gautheyrou, 2006). A single water extract (soil:extractant ratio 1:10 and 2 h of contact) was used to determine the pH and the contents of available phosphorus (P), potassium (K) and copper (Cu). The solution was centrifuged ($2000 \times g$ during 20 min at 20 °C) and filtered at 0.2 μm. The K and Cu contents were measured by flame atomic absorption spectrometry (Varian A600) and the P content was determined using the malachite green method (Ohno and Zibilske, 1991).

Soil microbial biomass carbon content (MBC) was quantified using the fumigation-extraction method (Wu et al., 1990). A TOC-V CSH (Shimadzu) carbon analyzer was used to measure organic carbon content in fumigated and non-fumigated samples. Soil respiration was measured according to ISO 14239 (1997); 10 g of dry soil were moistened to 100% of water holding capacity and then incubated for 28 days at 28 °C in the presence of 20 ml of 0.5 M NaOH solution. Emitted C-CO_2 combined with part of the NaOH, and the non-combined NaOH was titrated using a Titrino plus 848 titrator with 1 M HCl. The respiration flux was deduced by comparison with controls without soil. Three ratios related to the efficiency of substrate utilization were calculated: MBC/C_{org}, C-CO_2/C_{org} and the metabolic quotient (qCO_2), defined in this case as the amount of C-CO_2 respired (CO_2) over the 28-day incubation period divided by MBC.

Soil nematodes were extracted from 200 g of wet soil using the Oostenbrink elutriation method, together with sieving and cottonwood extraction (ISO 23611-4, 2007). Nematodes were fixed in a formaldehyde solution (4%) and a representative sub-sample was mounted on glass slides for identification at high magnification (400×). Around 150 nematodes per sample were identified to genus level and grouped into 5 trophic groups: Obligate Plant-Feeders (OPF), Facultative Plant-Feeders (FPF), Bacterial-feeders (Ba), Fungal-feeders (Fu) and Omnivores and Predators (Om-Pr). The combination of trophic groups and colonizer-persister (cp) value (Bongers, 1990) was used to classify each nematode taxon in a functional guild (Bongers and Bongers, 1998). Five nematode ecological indices were then calculated: Maturity Index (MI), Plant Parasitic Index (PPI) (Bongers, 1990), Enrichment Index (EI), Structure Index (SI) (Ferris et al., 2001) and Nematode Channel Ratio (NCR) (Yeates, 2003).

2.3. Statistical analyses

First, Principal Component Analyses (PCA) were performed separately on inherent soil parameters, dynamic chemical parameters and dynamic biological parameters in order to compare the distribution of plots and representative areas for these different sets of parameters. In order to assess the magnitude of variance ascribable to representative areas, between-class analyses (BCA) were performed for each set of parameters, followed with a Monte-Carlo test for result significance. Data concerning nematode trophic groups were transformed into proportions of total abundance to avoid a mass effect. All data were centered and scaled. We then performed a Redundancy Analysis (RDA) to determine the impact of use-invariant soil properties (inherent soil properties and representative areas) on dynamic soil parameters. To make the calculations possible, representative areas were coded as dummy variables, and data were centered and scaled. A post hoc, operational classification of soils, was proposed on the basis of the RDA analysis results (cf. Section 3 for details). Each plot was assigned to one of the six soil groups. Soil dynamic parameters

Table 1
Soil inherent and dynamic parameters characterized in the study (abbreviations and units).

Inherent soil parameters		
Coarse particles between 0.2 and 1 cm	P_2 mm	% (w/w) soil not sieved
Coarse particles >1 cm	P_1 cm	% (w/w) soil not sieved
Clay content	Clay	% (w/w) soil sieved at 2 mm
Silt content	Silt	% (w/w) soil sieved at 2 mm
Sand content	Sand	% (w/w) soil sieved at 2 mm
Total calcium carbonate content	$CaCO_{3\,tot}$	g kg^{-1} soil sieved at 2 mm
Actif calcium carbonate content	$CaCO_{3\,act}$	g kg^{-1} soil sieved at 2 mm
Dynamic soil parameters		
Chemical parameters		
pH		Without unit
Total soil organic carbon content	C_{org}	g kg^{-1} soil
Total soil nitrogen content	N_{tot}	g kg^{-1} soil
C/N		
Available P content	P	mg kg^{-1}
Available K content	K	mg kg^{-1}
Available Cu content	Cu	mg kg^{-1}
Effective cation exchange capacity	CEC_C	cmol^{+} kg^{-1}
Biological parameters		
Microbial biomass carbon	MBC	mg C kg^{-1}
Soil respiration	CO_2	µg g^{-1} over 28 days
Metabolic quotient	qCO_2	mg C_CO_2 g^{-1} MBC over 28 days
Carbon content from microbial biomass	MBC/C_{org}	Without unit
Respired Carbon to C_{org} ratio	C_CO_2/C_{org}	Without unit
Total nematode abundance	NemTot	ind. 100 g^{-1} soil
Proportion of obligate plant-feeding nematodes	OPF	% (ind/ind)
Proportion of facultative plant-feeding nematodes	FPF	% (ind/ind)
Proportion of bacterial-feeding nematodes	Ba	% (ind/ind)
Proportion of fungal-feeding nematodes	Fu	% (ind/ind)
Proportion of omnivores + predatory nematodes	Om_Pr	% (ind/ind)
Maturity Index	MI	Without unit
Plant Parasitic Index	PPI	Without unit
Enrichment Index	EI	Without unit
Structure Index	SI	Without unit
Nematode Channel Ratio	NCR	Without unit

were then compared between groups with ANOVAs or MANOVAs and Tukey post hoc tests. Data were square root or log transformed when necessary to satisfy normality criteria. All statistical analyses were conducted with the R software (R Core Team, 2012) using Vegan and Ade4 packages for multivariate analyses.

3. Results

3.1. Description of inherent and dynamic parameters and distribution of plots according to the 9 representative areas

Inherent soil parameters showed high contrast between representative areas (Table 2 and Fig. 2A). About two-thirds of vineyard plots were carbonated with total calcium carbonate contents ranging from 20 to 740 g kg^{-1} and reactive calcium carbonate contents from 0.55 to 16.70 g kg^{-1}. Carbonated soils were found in the areas of Aigues-Mortes, Montagnac, Saint-Hippolyte-du-Fort, Saint-Victor la Coste, Vergèze, and one plot from Faugères. Soils from Aigues-Mortes and Lesquerde were characterized by high sand content (respectively 88 and 81%) whereas sand contents varied between 17% and 52% for the other areas. The highest clay and silt contents were measured in soils from Montagnac, Saint-Hippolyte-du-Fort and Vergèze. The nine representative areas exhibited various degrees of stoniness. Soil particles with a size larger than 2 mm were present in plots from all representative areas except Aigues-Mortes. However particles larger than 1 cm (P_1 cm) were mainly found in soils from Jonquières-Saint-Vincent where they represented from 50 to 90% of topsoil mass. In some cases plots from different representative areas shared very similar inherent properties, as illustrated by Terrats and Faugères or by Montagnac and St-Hippolyte-du Fort (Fig. 2A). Between-class analysis revealed that 80% of the variance was ascribable to representative areas (Monte Carlo test $p < 0.001$).

Dynamic chemical parameters also varied considerably between plots (Table 2). Organic carbon content ranged from 3.9 to 64 g kg^{-1}. The mean values of C_{org} and N_{tot} amounted to, respectively, 13.25 and 1.07 g kg^{-1}. The C/N ratio had a mean value of 12.5, a minimum of 6.7 and a maximum of 29.3. CEC ranged between 1.4 and 24.7 cmol^{+} kg^{-1} and pH ranged between 4.99 and 8.6 (except for one plot whose higher pH of 9.11 suggested soil salinity or sodicity). Total P values ranged between 0 and 20 mg kg^{-1}, Cu contents between 0.08 and 1.29 mg kg^{-1} and K contents between 7.80 and 176.6 mg kg^{-1}. C/N ratios and pH showed relatively low variability, with coefficients of variation lower than 23% while those of water-extractable P, K and Cu ranged from 57.7 to 78.8%. Ordination of plots according to chemical parameters (Fig. 2B) revealed a distribution of plots along a first axis characterized by C_{org}, Cu and K contents and along a second axis characterized by CEC, pH and P content. These two axes accounted for 60% of the total variance. The segregation of the representative areas using chemical dynamic parameters was less obvious than using inherent parameters and occurred mainly along the second axis. Likewise, BCA revealed that 46% of the variance was ascribable to representative areas (Monte Carlo test $p < 0.001$).

The 164 plots also differed considerably in their biological parameters (Table 2). Means of microbial biomass carbon (MBC) and 28-day soil respiration amounted to 78 mg C kg^{-1} soil and 1.19 µg C g^{-1} soil respectively, with high coefficients of variation (64.7 and 71.3%). The MBC/C_{org} ratio varied from 0.18 to 1.15, the metabolic quotient (qCO_2) from 0.04 to 6.52 mg C_CO_2 g^{-1} MBC and the CO_2/C_{org} ratio from 0.07 to 26.24. The largest coefficient of variation was found for the metabolic quotient (134.5%), followed by the CO_2/C_{org} ratio (98.7%), while the MBC/C_{org} ratio showed a coefficient of variation of 35.7%. Mean total nematode abundance was 857.5 ind. 100 g^{-1} dry soil with a coefficient of variation of 70.7%. The percentages of the different trophic groups of nematodes were also highly variable, especially for obligate plant-feeding nematodes and the group of omnivores and predators. Plant parasitic nematodes averaged 16% and ranged from 0 to 62% while omnivores and predators averaged 9% and ranged from 0 to 47%. Bacterial and fungal-feeding nematodes averaged respectively 29.5% and 26.5% of the nematode community. In most of the plots, EI was below 50, whereas SI ranged between 5 and 88 with a mean equal to the median around 50. Mean MI and PPI were respectively 2.39 and 2.33 and similar to the median values. The NCR index displayed the same values for mean and median at 0.53. The nematode indices displayed lower coefficients of variation (especially MI and PPI) than nematode abundances. The two first axes of the canonical representation of plots according to biological parameters explained 42% of total variance (Fig. 2C). Parameters mainly contributing to site dispersion along the two first axes were those relative to nematofauna: MI and SI indices (loaded on the negative and positive values respectively on axes 1 and 2), total number of nematodes (NemTot) and proportion of obligate plant-feeding nematodes (OPF) (negative values on both axes 1 and 2), and

Table 2
Mean and distribution parameters of the 31 parameters in the 164 studied vineyard plots. For the abbreviation of the parameters see Table 1. ND = not detected.

	Mean ± standard deviation	Median	Minimum	Maximum	Coefficient of variation
Soil inherent parameters					
P_1 cm (%)	13.7 ± 23.3	4.7	0	90.0	169.8
P_2 mm (%)	17.8 ± 14.9	16.8	0	57.8	83.3
Clay (%)	22.1 ± 14.0	17.6	3.6	68.5	63.2
Silt (%)	28.9 ± 13.2	31.8	2.0	60.5	45.7
Sand (%)	49.0 ± 23.8	46.2	5.6	93.3	48.6
Tot_$CaCO_3$ (g kg^{-1})	157.1 ± 178.2	111.0	ND	714.0	113.4
Act_$CaCO_3$ (g kg^{-1})	3.4 ± 4.3	0.8	ND	16.7	128.4
Soil dynamic parameters					
pH	7.6 ± 0.96	8.1	5.0	9.1	12.7
C_{org} (g kg^{-1})	13.2 ± 6.3	11.9	3.9	64.0	48.1
N_{tot} (g kg^{-1})	1.07 ± 0.44	1.03	0.28	4.19	40.8
C/N	12.5 ± 2.8	12.7	6.7	29.3	22.2
P (mg kg^{-1})	3.8 ± 3.0	3.2	0	20.0	78.8
K (mg kg^{-1})	44.1 ± 25.4	37.5	7.8	176.6	57.7
Cu (mg kg^{-1})	0.41 ± 0.26	0.35	0.08	1.29	63.8
CEC_C ($cmol^{+}$ kg^{-1})	10.4 ± 5.7	9.6	1.4	24.7	54.4
MBC (mg C kg^{-1})	78.0 ± 50.4	69.7	8.2	413.5	64.7
CO_2 (μg g^{-1})	1.19 ± 0.85	1.08	0.02	5.02	71.3
qCO_2 (mg C_CO_2 g^{-1} MBC over 28 days)	0.52 ± 0.7	0.35	0.04	6.52	134.5
C_CO_2/C_{org}	2.62 ± 2.59	2.13	0.07	26.24	98.7
MBC/C_{org} (%)	0.57 ± 0.2	0.56	0.18	1.15	35.7
NemTot (ind. 100 g^{-1} soil)	858 ± 607	760	36	3638	70.7
OPF (%)	16.3 ± 13.4	13.8	0	62.3	82.7
FPF (%)	19.1 ± 11.2	17.5	0.7	50.3	58.5
Ba (%)	29.5 ± 12.8	28.5	4.7	67.4	43.4
Fu (%)	26.5 ± 13.0	25.1	3.3	80.0	49.0
Om_Pr (%)	8.7 ± 5.6	7.8	0	47.3	64.9
MI	2.39 ± 0.28	2.35	1.80	3.59	11.6
PPI	2.33 ± 0.24	2.28	2.00	3.03	10.5
EI	40.2 ± 10.6	40.4	6.3	75.9	26.3
SI	50.1 ± 17.7	50.4	5.9	88.7	35.4
NCR	0.53 ± 0.16	0.53	0.15	0.88	31.0

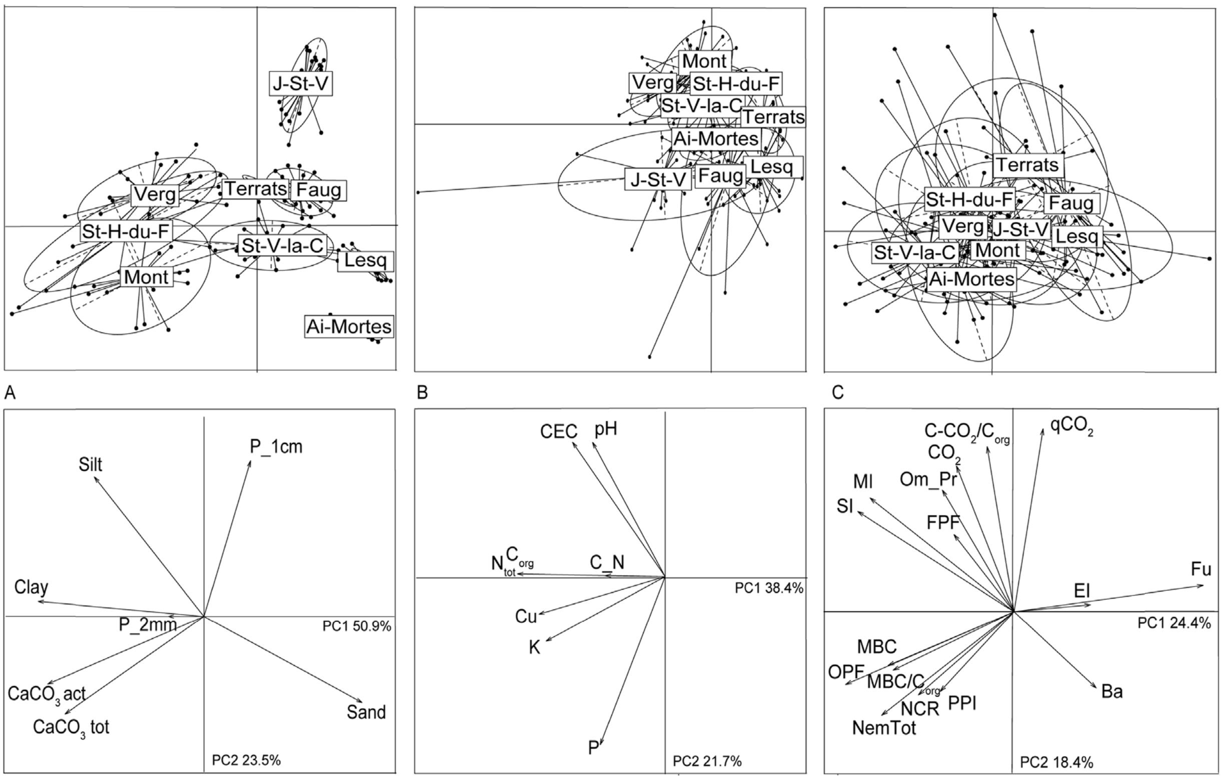

Fig. 2. Principal component analyses (PCA) performed with the different sets of parameters. (A) Inherent soil parameters; (B) chemical dynamic parameters; (C) biological dynamic parameters. Above: projection of plots and representative areas; below: projection of parameters. For parameter abbreviations see Table 1. Ellipses highlight the nine representative areas: Aigues-Mortes (Ai-Mortes), Faugères (Faug), Jonquières-Saint-Vincent (J-St-V), Lesquerde (Lesq), Montagnac (Mont), Saint-Hippolyte-du-Fort (St-H-du-F), Saint-Victor-la-Coste (St-V-la-C), Terrats (Terrats), Vergèze (Verg).

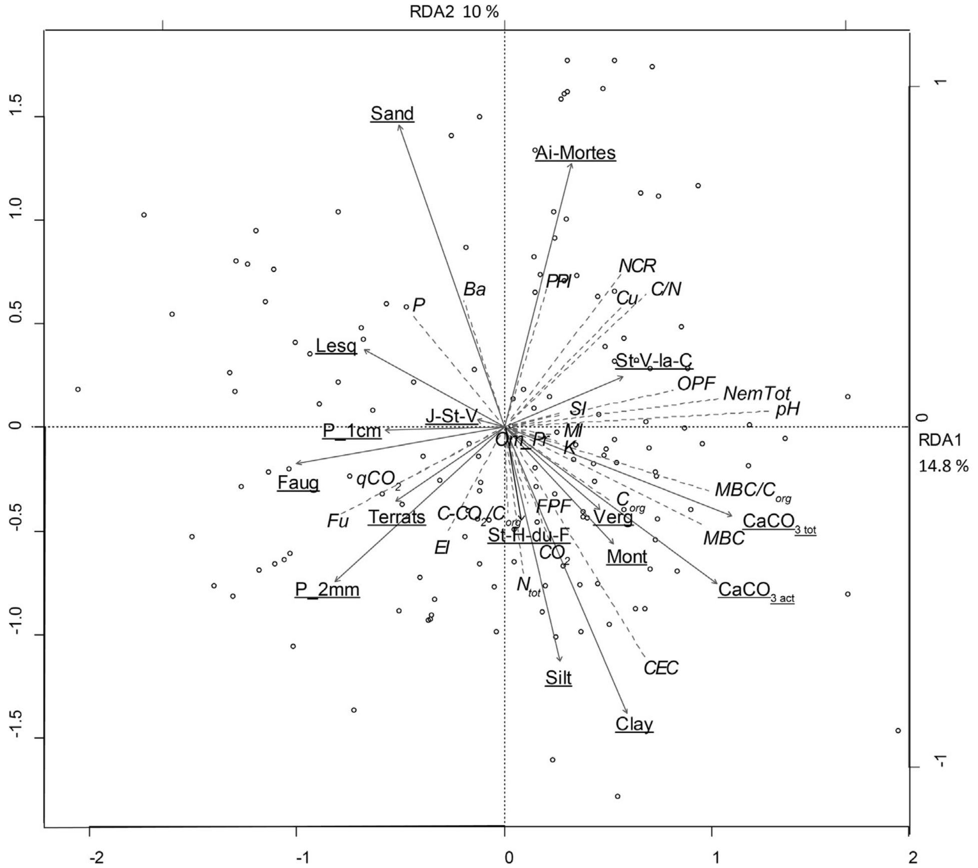

Fig. 3. RDA triplot of dynamic indicators (dotted arrow and italic text) explained by inherent indicators and representative areas (continuous arrow and underlined text). Open dots represent the plots. For the abbreviation of soil indicators, see Table 1. Abbreviations of the nine representative areas are: Aigues-Mortes (Ai-Mortes), Faugères (Faug), Jonquières-Saint-Vincent (J-St-V), Lesquerde (Lesq), Montagnac (Mont), Saint-Hippolyte-du-Fort (St-H-du-F), Saint-Victor-la-Coste (St-V-la-C), Terrats (Terrats), Vergèze (Verg).

fungal-feeding nematodes (Fu) (positive value on axis 1). The metabolic quotient and CO_2/C_{org} ratio also contributed (positive value on axis 2). Although canonical ordination did not clearly discriminate between areas, BCA analysis revealed that 24% of biological parameter variance was ascribable to representative areas (Monte Carlo test $p = 0.001$).

3.2. *Influence of representative areas and inherent parameters on dynamic parameters*

The first two canonical axes of the RDA significantly explained 25% of the variance (Fig. 3). Nematode abundance (NemTot), microbial biomass carbon (MBC), MBC/C_{org}, pH, proportion of obligate plant-feeding nematodes (OPF), and C_{org} content were positively influenced along the first axis by calcium carbonate content and areas of Saint-Victor-la-Coste, Vergèze and Montagnac. Conversely, high proportions of fungal-feeding nematodes (Fu) and high metabolic quotients (qCO_2) were found in sites without calcium carbonate and with particles larger than 2 mm. Areas of Lesquerde, Faugères and Terrats also contributed to the formation of this first axis. The second axis revealed a textural gradient with clay and silt contents positively influencing CEC, N_{tot}, soil respiration, $C\text{-}CO_2/C_{org}$ and to a lesser extent the proportion of facultative plant-feeding nematodes. On the other side of the axis, sand content positively influenced bacterial-feeding nematodes and available P content. Note that the Aigues-Mortes area also highly contributed to this axis and strongly influenced the PPI. The NCR, Cu content and C/N ratio were positively explained by this second canonical axis. The other parameters such as SI and MI, K content and proportions of omnivore + predator nematodes showed shorter projections, indicating that their variations were not strongly influenced by area and/or by inherent soil properties. The area of Jonquières-Saint-Vincent did not contribute to explaining the dynamic parameters in the canonical ordination. However this representative area and soil particles larger than 1 cm contributed significantly to a third axis (data not shown) which explained a part of the variance of available P content and bacterial-feeding nematode relative abundance.

3.3. *Operational classification of soils*

The RDA analysis confirmed that most of the dynamic parameters were basically affected by the calcium carbonate content and textural properties of topsoils, which characterized and discriminated most of the representative areas. We proposed on this basis to classify the 164 vineyard plots into six soil groups based on texture classes using the FAO triangle, and active calcium carbonate content (presence or absence). We decided to separate all Jonquières-Saint-Vincent vineyards as an entire group given that these soils were characterized by a large proportion of stones. Parameters measured on 2 mm-sieved samples of such stony soils, with very specific water and heat transfer characteristics, may thus not be representative of whole soil functioning. Soil groups are presented in Table 3. Three of these groups corresponded to a single representative area (groups 1, 4 and 6) whereas groups 2 and 3, and to a lesser extent group 5 contained plots from different

Table 3
Characterization of the 6 soil groups according to inherent indicators and pH (mean standard deviation). The representative areas from which plots originated are specified as well as the number of plots.

Group	Texture (FAO)	Carbonate status	P_1 cm (%)	P_2 cm (%)	pH	Number of plots per area	Total number of plots
1-Alkaline sandy	Coarse	Carbonated	0.0 ± 0.1	0.9 ± 0.5	8.5 ± 0.2	Aigues-Mortes (18)	n = 18
2-Alkaline fine textured	Fine	Carbonated	3.8 ± 4.8	25.5 ± 15.2	8.2 ± 0.1	Montagnac (10), Vergèze (8), St-Hippolyte-du-Fort (11), Faugères (1)	n = 30
3-Alkaline medium textured	Medium	Carbonated	2.4 ± 2.6	13.2 ± 11.4	8.2 ± 0.2	Montagnac (11), Vergèze (9), Saint-Victor-la-Coste (23), Saint-Hippolyte-du-Fort (4)	n = 47
4-Coarse mildly acidic	Coarse	Non-Carbonated	12.9 ± 3.6	31.8 ± 3.8	6.5 ± 0.9	Lesquerde (19)	n = 19
5-Mildly acidic medium textured	Medium	Non-carbonated	13.2 ± 7.6	29.6 ± 6.0	6.5 ± 0.8	Terrats (11) and Faugères (20)	n = 31
6-Neutral stony	Stony soils	Non-carbonated	72.1 ± 22	0.0 ± 0.0	7.3 ± 0.5	Jonquières-Saint-Vincent (19)	n = 19

representative areas. Descriptive statistics (boxplots) of dynamic parameters according to soil groups are given in Figs. 4 and 5. The ANOVA or MANOVA results showed significant differences between soil groups for all parameters except for qCO$_2$, C-CO$_2$/C$_{org}$ and the proportion of omnivores + predators (Om_Pr). Nematode indices showed weak differences between groups in comparison with other dynamic parameters: due to their construction which takes into account the relative abundance of soil animals belonging to different functional groups, they are more independent of soil characteristics than most of the other biological parameters. Interestingly parameters such as C$_{org}$, MBC, N$_{tot}$, CO$_2$, and MBC/C$_{org}$ showed similarities in their response patterns to soil groups. Note that some of these parameters were highly correlated, namely MBC and C$_{org}$ (r = 0.84), MBC and N$_{tot}$ (r = 0.78), MBC and CEC (r = 0.65).

4. Discussion

4.1. The soil-group approach for soil quality evaluation

Our study highlights the complementarity of studying inherent as well as chemical and biological dynamic soil parameters when assessing soil quality, as illustrated by the different distributions of vineyard plots obtained with the different sets of parameters (PCAs in Fig. 2). Part of the variation in measured dynamic properties (25%) was significantly explained by inherent properties, with different sensitivities according to the dynamic parameter considered.

These findings support the statements made by Herrick (2000) and more recently by Pulleman et al. (2012), who pointed out the importance of taking into account inherent soil properties for

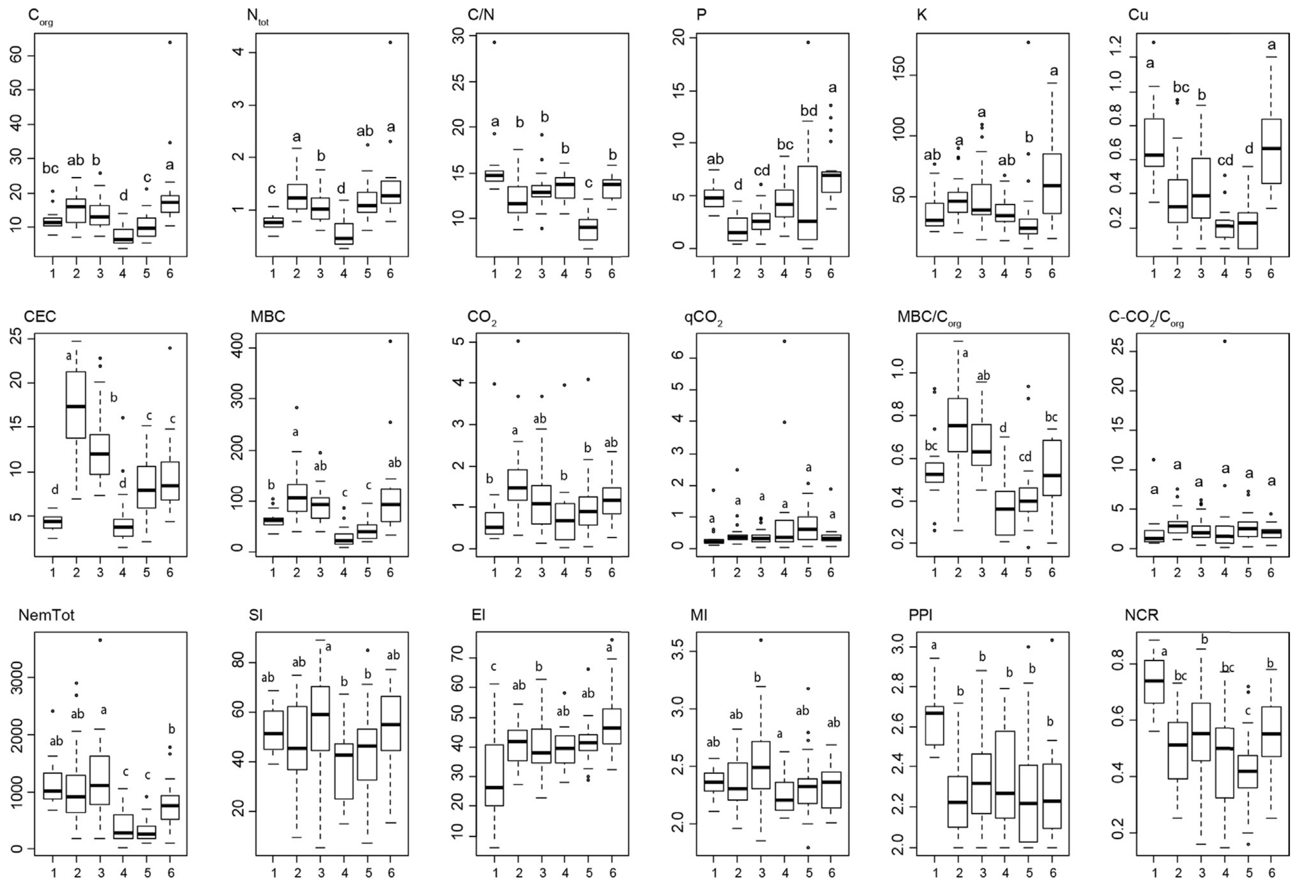

Fig. 4. Boxplots of the dynamic soil parameters according to soil groups. 1-Alkaline sandy soils, 2-alkaline fine textured soils, 3-alkaline medium textured soils, 4-coarse mildly acidic soils, 5-mildly acidic medium textured soils, 6-neutral stony soils. Letters are the results of a Tukey HSD post hoc test following an ANOVA. For the abbreviations and units of the parameters, see Table 1.

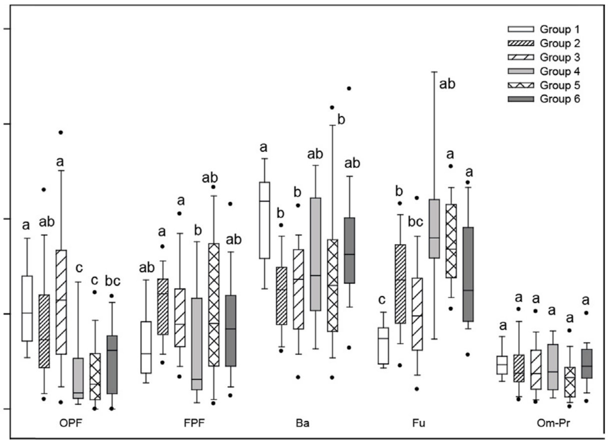

Fig. 5. Boxplots of the proportion of the different trophic groups of nematodes according to soil groups. 1-Alkaline sandy soils, 2-alkaline fine textured soils, 3-alkaline medium textured soils, 4-coarse mildly acidic soils, 5-mildly acidic medium textured soils, 6-neutral stony soils. Letters are the results of a Tukey HSD post hoc test following a MANOVA. For trophic group abbreviations see Table 1.

the correct interpretation of dynamic parameter values. A part of the remaining measured variation in dynamic soil parameters is expected to be explained by management practices which would be easier to evaluate within a given soil group. On this basis, we defined groups of Mediterranean vineyard soils with different potential functioning, as Rutgers et al. (2008) did at the Netherlands scale. Our soil group approach offers the possibility of monitoring the effect of wine-growing practices on vineyard sustainability while taking into account the specificities and the potential of *terroirs* as defined in the present paper. Viticulture practices may not be evenly represented in each soil group, meaning that some effects attributed to inherent soil parameters may be partly linked to vineyard management.

Our six soil groups were based on calcium carbonate content, texture and stoniness rather than on soil classification as only the properties of the topsoil (0–15 cm) were considered. Soil classification requires a complete soil profile analysis in pits frequently dug to a 1 or 2 m depth. It is recommended as part of the process of planting a new vineyard, in order to take whole-profile inherent soil quality into account in the selection of sites suitable for different land uses (e.g. Mueller et al., 2007) or of appropriate vine varieties and rootstocks. Focusing only on the topsoil is nevertheless justified in our case, as we are ultimately interested in characterizing dynamic soil quality in relation to management practices, which can be expected to have the strongest impact on the topsoil layer. Winegrowers point out the lack of advice from consultants about soil quality management with respect to their *terroir* specificities (Coll et al., 2012b); a significant part of these characteristics correspond with the inherent soil parameters we measured. Our approach is a reasonable trade-off for winegrowers, taking into account analysis cost and knowledge requirements; it could plausibly be applied within a framework of regular monitoring of soil quality for viticultural decision-making.

In contrast with Karlen et al. (2003), we did not consider the pH as an inherent soil property. The pH buffer capacity in soils arises from dissolution/precipitation of aluminosilicates or calcium carbonates at low and high soil pH values respectively, but for medium pH buffer capacity arises from protonation/deprotonation reactions on variable mineral and organic surface charges (Aitken and Moody, 1994). In the latter case, the pH can change rapidly due to proton addition or loss associated with the transformation and cycling of elements (C, N), or due to liming practices. The choice of pH as an inherent or dynamic parameter is thus a matter of debate, like the choice of organic carbon in other studies (Karlen et al., 2003).

Also, a survey of management practices (data not shown) revealed that some winegrowers practiced liming. We therefore decided to classify pH as a dynamic indicator even though it is relevant only for non-carbonated soils.

Despite the above-mentioned biases, we believe that our soil groups are relevant for wine growers who commonly measure the inherent parameters we used, and who can therefore easily assign their soil to the correct group.

4.2. Relationships between inherent and dynamic parameters

In our study, C_{org} and N_{tot} were significantly influenced by texture and calcium carbonate contents. Hassink (1994, 1997) elucidated the relationships between texture and organic matter but the relationship between $CaCO_3$ and organic matter was less obvious. Following Bronick and Lal (2005), we hypothesize that Ca^{2+} favors physical protection of organic matter in these soils by promoting soil aggregation (Puget et al., 2000; Six et al., 2004). Concerning CEC, as expected (van Erp et al., 2001) we measured the lowest values in sandy soils depleted in organic matter (groups 1 and 4), and the highest values in the more clayey soils exhibiting a higher soil organic matter content (group 2). These results suggest that a larger beneficial effect of organic matter application or cover crops is expected in groups 1 and 4 than in other soils. Water extractable Cu contents were of the same order of magnitude as values reported for Cu-contaminated soils (Courchesne et al., 2006; Michaud et al., 2007) and for other conventional and organic vineyards located in Languedoc-Roussillon (Coll et al., 2011). This observation is the result of the use of large amounts of Cu-based sprays such as the Bordeaux mixture to prevent fungal diseases (Chaignon et al., 2003). We could have expected to find the lowest values for groups 2 and 3 as organic matter and high pH are known to limit Cu availability (Sauvé et al., 2000). However, a number of studies in vineyard soils have shown a positive correlation between soil organic matter content and Cu contamination (e.g. Chaignon et al. (2003) in some Languedoc-Roussillon vineyards). These results stress the need to consider other extraction procedures to correctly interpret Cu availability and to obtain a broader evaluation of Cu contamination and its toxicity for organisms as pointed out by Komarek et al. (2010). We propose a similar conclusion concerning the evaluation of P or K availability. Complex geochemical processes involved in the availability of these nutrients would require more than one extraction procedure to establish an effect of inherent soil properties on the availability of these nutrients for plants. In addition, considering only the topsoil properties may not be relevant for assessing the nutrition of deep-rooted plants such as *Vitis* spp.

Microbial parameters varied consistently with previously published results. Microbial biomass carbon displayed the same response pattern between soil groups as C_{org}. A high correlation between these two parameters has already been observed in many studies (Dequiedt et al., 2009; Fierer et al., 2009), highlighting their redundancy for large scale datasets. Among the three ratios proposed to assess the efficiency of substrate utilization (MBC/C_{org}, qCO_2 and CO_2/C_{org}), the MBC/C_{org} ratio was significantly affected by carbonates as well as by texture, with maximum values in clayey soils. This result contradicts a study by Sparling (1992) who found higher MBC/C_{org} ratios in sandy soils than in fine-textured soils. However our values of MBC/C_{org} were systematically distributed at the lower end of the range of values found by Sparling (1992), and we can suggest that their hypothesis of a protection by clay minerals of organic matter against mineralization may not be generalizable to our soil conditions. Moreover, the Mediterranean climate of our site is characterized by drought periods that may favor microbial activity in clayey soils, at the expense of organic matter content. Clayey soils retain more moisture (Satish and Dong, 2007) and consequently may offer a more favorable habitat for microorganisms than sandy soils in drought-stressed environments. These results stress the relevance of the regional approach and we agree with Sparling (1992) that values of the MBC/C_{org} index should only be interpreted within similar soil type and climate zones. Probst et al. (2008) as well as Wardle and Ghani (1995) reported an increase in $q\mathrm{CO_2}$ with decreasing pH, clay content and microbial biomass carbon. Although we found no significant difference in mean $q\mathrm{CO_2}$ between soil groups, we noted different ranges of variation. The $q\mathrm{CO_2}$ parameter displayed high variation in groups 4 and 5 whereas it remained stable in the other soil groups. Assessing $q\mathrm{CO_2}$ as an indicator of soil quality would be relevant only in soils displaying inherent properties similar to those of groups 4 and 5 (both non carbonated). One should note that CO_2 emission originating from calcium carbonate dissolution can occur in calcareous soils (Bertrand et al., 2007; Emmerich, 2003; Tamir et al., 2011); and CO_2 respired in closed-chamber protocols can also re-precipitate in carbonated soils (Oren and Steinberger, 2008). In addition, Dong et al. (2013) highlighted that the overestimation of soil respiration varied depending on the type and amount of carbonate present in the soil. For these reasons we believe this parameter is not a suitable indicator for carbonated soils, except for comparisons within a group of soils that present similar carbonate characteristics and concentrations.

Studying nematode communities provided further information corroborating different soil functioning between soil groups. The difference in nematode abundance between groups 4 and 5 and groups 1, 2, 3 and 6 was probably due to a pH effect as pointed out by Mulder et al. (2005) and Popovici and Ciobanu (2000). Like Liskova et al. (2008) we observed a change in the trophic structure of the nematode community according to soil groups. Whereas the proportion of fungal-feeding nematodes was highest in mildly acid soils (groups 4 and 5), the proportion of obligate plant-feeders was highest in carbonated soils (groups 1, 2 and 3). The first result was probably due to a change in the bacteria/fungi ratio with decreasing pH (Bååth and Anderson, 2003). The second result corroborates findings by Rahman et al. (2007), who observed a decrease in plant parasitic nematodes with decreasing pH. This highlights the possibility of a higher nematode phytopathogenic stress on vineyards in carbonated soils and especially in sandy soils of group 1, which also exhibited the highest PPI (higher proportion of plant parasitic nematodes). However this phytopathogenic stress has to be confirmed by the identification of nematodes at the species level given that the main plant-parasitic nematode associated with vineyards is *Xiphimena index* and, according to Sultan and Ferris (1991), the survival of this species is low in sandy soils. The higher proportion of bacterial-feeding nematodes in the sandy soils from group 1 (which logically also displayed the highest NCR) than in soils from groups 2 and 3 pointed out differences in the soil foodweb and decomposition pathway according to soil texture. Despite the methodological constraints associated with their identification, nematodes appear to be good indicators of soil food web and decomposer community structure because of the comprehensive information provided by their analysis. Moreover, nematofaunal indices (SI and EI) were not significantly influenced by the soil type whereas they were significantly influenced by management practices (Villenave et al., 2013; companion paper in prep); so they can be compared between soils whatever the inherent characteristics of these soils.

With dynamic soil parameter distributions tabulated by class of inherent soil properties, we believe that stakeholders can easily appraise their topsoil quality. We chose not to specify threshold benchmark values related to a "good" soil functioning, as did Rutgers et al. (2008) for different land uses and soil types in the Netherlands. Defining thresholds for each soil parameter implicitly assumes additivity and equal importance of all parameters, while they are in fact interrelated in different ways and their relative importance can depend on winegrower priorities. Coll et al. (2011) demonstrated that changes in vineyard practices could improve some parameters while negatively affecting others. As a consequence, it may be difficult to define practices that improve all soil functions simultaneously.

4.3. Global vineyard topsoil quality in the Languedoc-Roussillon region

Studying the range of values of dynamic parameters provides information about some trends in global topsoil quality in Mediterranean vineyards as assessed in the Languedoc-Roussillon region. The most relevant result is the low organic matter content in these soils. Even though we measured C_{org} contents up to 64 g kg^{-1}, the median value was 11.9 g kg^{-1}, which can be qualified as low. Moreover, a quarter of the 164 plots contained less than 10 g kg^{-1}, which is, according to Jones et al. (2004), a threshold value of C_{org} for soil erosion risk. Improvement of the organic status of these soils must therefore be promoted (Le Bissonnais et al., 2007) and practices minimizing losses of soil carbon should be considered (Carlisle et al., 2006; Steenwerth et al., 2010). Biological parameters such as MBC, nematode abundance or the MBC/C_{org} ratio indicated a low biological activity in these soils and a poor resource utilization efficiency in comparison with other crops or pastures (Carter, 1991; Sparling, 1992; Villenave et al., 2013), or with other vineyards under different climatic conditions (Probst et al., 2008). Moreover the low Enrichment and Maturity indices (EI and MI) obtained from nematode faunal analysis indicated poor resource availability as well as disturbance or contamination of the soil. This result corroborates studies by Coll et al. (2011) and Manachini (2001) in other vineyards in Mediterranean regions. However, our findings also pointed out that vineyard soils in the Languedoc-Roussillon region were not systematically characterized by low SI values indicative of a degraded soil food web. As a consequence of this survey, we can consider that Languedoc-Roussillon vineyards generally exhibit low topsoil quality regarding resources and the intensity of biological activity but not necessarily regarding its functioning through soil food web structure. Mildly acidic and/or sandy soils displayed the lowest soil quality focusing both on biological and organic status and the efficiency of microbial activity. This suggests that these soils may be more vulnerable and less resilient to perturbations. It is necessary, especially in these soils, to look for and promote management practices that improve biological activity and organic matter content (Steenwerth and Belina, 2008) as well as the efficiency of substrate utilization.

5. Conclusion

Most of the dynamic parameters were strongly affected by inherent soil properties, emphasizing the need to consider the inherent potential of different soils in assessing their quality and evaluating management practices. For this reason we proposed a soil classification based on stoniness, carbonate content and texture for the appraisal of topsoil quality using various indicators. We underlined the importance of using appropriate indicators for a holistic approach toward topsoil quality in a specific *terroir*. Completing those indicators of topsoil functioning with indicators of erosion risk, such as aggregate stability, would nevertheless be necessary to assess topsoil quality in a more comprehensive manner, and to struggle against the main threats of these vulnerable soils. This study was conducted in vineyards of the Languedoc-Roussillon in the Mediterranean part of France but could be reproduced for any other land use type and climatic region, under the condition that a sufficient database is produced to reveal structural trends. Baselines provided by such studies would allow stakeholders to compare their topsoil quality even with minimum knowledge in soil sciences. The evaluation of topsoil quality can reveal endangered soil functions and thus provide farmers with guidelines for the prioritization and adaptation of farming practices allowing sustainable soil management.

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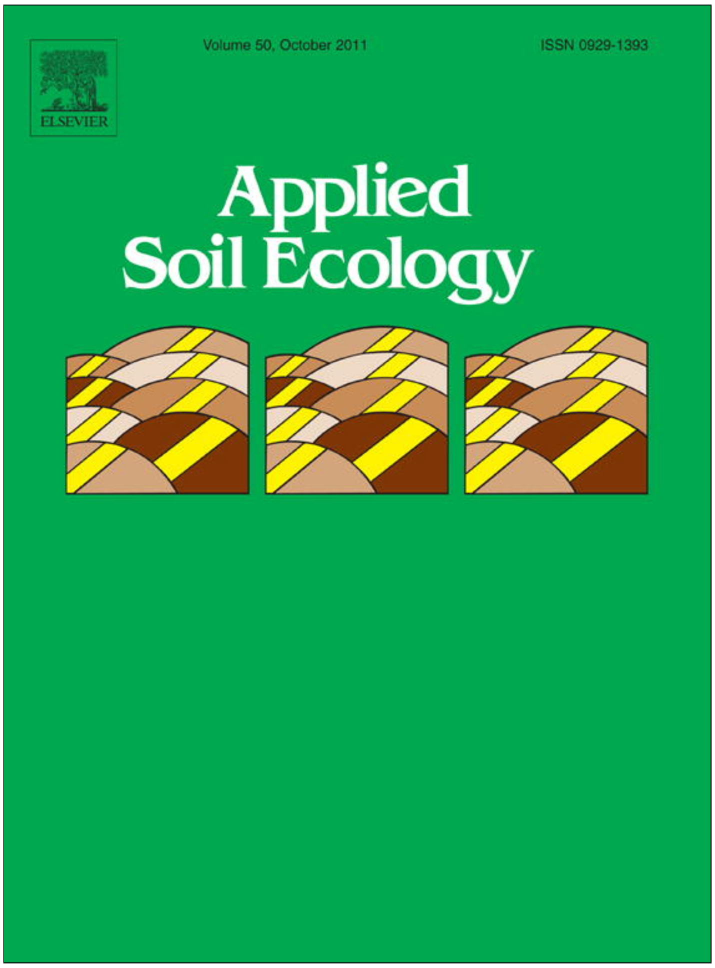
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Organic viticulture and soil quality: A long-term study in Southern France

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ABSTRACT

The rate of conversion of conventional vineyards into organic farming is currently increasing. This results in modifications of agricultural practices such as the application of organic manure, the use of tillage or grass-cutting to control weeds and the application of natural pesticides with preventive action. One of the aims of organic farming is to preserve the environment. In this context, the objective of our work was to evaluate the long-term effects of organic viticulture on soil quality. The study was conducted in a commercial vineyard where plots which had been organically managed for 7 (Organic7), 11 (Organic11) and 17 years (Organic17) were compared to conventionally managed plots (Conventional). Soil physical and chemical parameters (bulk density, organic matter, available phosphorus, potassium and copper contents) and biological parameters (soil microbial biomass, density of nematode trophic groups and density and biomass of earthworm ecological categories) were measured. The organic farming led to an increase in soil organic matter, potassium content, soil microbial biomass, plant-feeding and fungal-feeding nematode densities. However, organic farming increased soil compaction, decreased endogeic earthworm density and did not modify the soil micro-food web evaluated by nematofauna analysis. Our study highlights the difficulty to show the benefits of organic farming on global soil quality in this particular pedoclimatic area and set of farming practices.

1. Introduction

For food and beverage safety issues, environmental considerations and economic interests are prompting more and more winegrowers to convert conventionally managed vineyards into organic farming. From 2001 to 2008, the area of organically managed French vineyards increased by 110%: 13,426 ha in 2001 (AgenceBIO, 2002) and 28,190 ha in 2008 (AgenceBIO, 2009). Conventional viticulture uses agrochemicals such as manufactured inorganic fertilizers and synthetic chemical pesticides. In contrast, these are banned in organic farming, while only organic fertilizers, crushed rocks and a few non synthetic pesticides are allowed (Briar et al., 2007). Instead of applying herbicides, weeds are managed by tillage or grass-cutting in organic farming. As a consequence, organic farming claims to reduce disturbance intensity of agricultural practices on the environment (Reganold et al., 1987), and especially on soil. Indeed, soil is a non-renewable resource and most vineyard soils are considered as highly degraded in terms of loss of organic carbon as a result increasing erosion and diminution of nutrient contents (Le Bissonnais et al., 2007; Martinez-Casasnovas and Ramos, 2009), accumulation of metals and organic pollutants (Chaignon et al., 2003; Komarek et al., 2010) or compaction due to tractor traffics (Coulouma et al., 2006). One of the main objectives of organic farming is to give more importance to soil biological functioning in order to improve its physical (affecting the circulation of water, aeration), chemical (affecting the availability of nutrients) and biological (affecting the biodiversity and fate of organic matter) properties (Van Bruggen and Semenov, 2000). Furthermore, in wine production, the soil is considered, together with climate, as a key component of Terroir (Van Leeuwen et al., 2004) which can influence the wine quality (Van Leeuwen and Seguin, 2006).

As defined by Doran and Parkin (1994) "the soil quality is the ability of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health". Soil quality is generally evaluated by the interpretation of physical, chemical or biological indicators. Among physical and chemical indicators, those most used by winegrowers are soil bulk density, pH, availability of major nutrients (N, P, K, Ca, Mg), organic matter content which is known to increase soil water holding capacity (Teixeira et al., 2011), to promote soil aggregation (Le Bissonnais et al., 2007; Morlat and Chaussod, 2008) and to constitute a pool of available nutrients (Haynes, 1999). Biological indicators or bioindicators are considered to give an evaluation of

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soil functioning because soil organisms have intimate relationships with their surroundings (Franzle, 2006), and then can give information about soil degration or improvement (Bispo et al., 2011). The most widely studied are microorganisms and soil fauna (Huber et al., 2008; Bispo et al., 2011). Microorganisms are involved in different key processes in the ecosystems, such as the decomposition of organic matter, humus formation, soil aggregation, retention and cycling of nutrients, and various symbiotic and parasitic relationships with plants (Paul and Clark, 1996). Other useful bioindicators are nematodes and earthworms. To evaluate the soil food web, the soil nematodes are pertinent bioindicators because they present an important diversity of trophic groups such as plant-feeders, bacterial-feeders, fungal-feeders, omnivores and predators (Yeates et al., 1993; Villenave et al., 2004). Indeed, they are implicated in soil organic matter mineralization processes (Neher, 2001). In addition, they are ubiquitous and thus present in all pedo-climatic situations including habitats that vary from pristine to extremely degraded (Bongers and Ferris, 1999). Other representatives of soil fauna are earthworms. They are described as bioindicators of soil quality as they modify soil physical properties. In particular they maintain soil structure and modify soil hydrodynamic functioning (Eijsackers et al., 2005). They provide additional information about the fate of organic matter. As a consequence both nematodes and earthworms give different and therefore complementary data on soil functioning.

Several authors compared vineyard soil characteristics after inorganic and organic fertilizer application (Morlat and Chaussod, 2008; Bustamante et al., 2011) and under different grass management practices (Monteiro and Lopes, 2007; Smith et al., 2008; Steenwerth and Belina, 2008). Until now, scientific knowledge concerning the effects of organic viticulture on soil functioning is scarce, except the studies of Reeve et al. (2005), Probst et al. (2008) and Reinecke et al. (2008). There is thus a great need to evaluate if soil quality is altered by changing practices during the conversion of vineyards into organic farming. Former studies have focused on earthworms and microbial biomass (Reuter and Kubiak, 2003; Ingels et al., 2005; Whitelaw-Weckert et al., 2007), but fewer studies were conducted on nematodes as bioindicators of vineyard soil quality (Sanchez-Moreno and Ferris, 2007; Rahman et al., 2009). To our knowledge, none of this research combined several physical, chemical and biological indicators to give a complete overview of soil quality of vineyards.

In this work, we measured the long-term effects of organic viticulture by physical and chemical indicators (organic matter content, availability of major nutrients and contaminant (Cu), bulk density) as well as by bioindicators (microbial biomass, nematodes and earthworms). With our methodology, we can then evaluate if intensive practices denoted as conventional are more damaging to soil quality and biological functions than are arguably organic practices. The present study was conducted on 24 vineyard plots: 10 were conventionally managed, while the others had different ages of conversion into organic farming (7, 11 and 17 years).

2. Material and methods

2.1. Site description, management and field plot design

This study was conducted in May 2009 in Cruscades, which is located in the South of France, in the Languedoc-Roussillon region (43°11′29.13″N, 2°49′1.78″E; 26–50 m elevation). The climate is typically Mediterranean with 14.7 °C mean annual temperature, 600 mm of annual rainfall and 1380 mm of annual ETP Penman-Monteith (average value based on data collected from 2000 to 2010 by Météo-France). The plots did not present any slope. The soil was silty-clay, with 42 ± 2% of silt, 36 ± 1% of clay and 22 ± 2% of sand. It was calcareous (208 ± 7 g of total CaCO3 kg^{-1}), yielding a pH in water of 8.3. The soil water-holding capacity was 20.6 ± 0.5% (w/w).

The study was conducted on 24 commercial wine grape vineyard plots whose mean area was around 1.5 ha. They presented different varieties of grape (*Vitis vinifera* L.) such as Cabernet-Sauvignon, Carignan N, Chardonnay, Cinsault, Grenache N, Merlot, Mourvèdre, Pinot N and Syrah. The rootstocks were mainly R110 and R140, but Riparia and 410a were also present. The year of plantation varied from 1932 to 2003. The plantation density was comprised between 3300 and 5000 vines per hectare. Ten plots were managed according to conventional farming (Conventional) and the others according to organic farming (Organic). Five plots have been organically managed since September 2001, officially certified in 2004 (Organic7). Four plots have been organically managed since September 1997 (Organic11) and the last 5 plots since September 1991 (Organic17). These 4 sets of plots will be referred to as treatments here below. Conventional agricultural practices were identical for each treatment before the organic conversion as well as organic agricultural practices after the conversion (Table 1). Four representative subplots (5 vines × 4 inter-rows) per plot were sampled. Consequently, 96 subplots were studied.

2.2. Sampling procedure

The sampling was conducted in springtime, a few days after mild raining events and could be considered to occur within the same time frame (on May 2009, from 4th to 15th for earthworms and from 22nd to 28th for soil). The soil water content was 14.7 ± 0.3% (w/w) for earthworm sampling and 11.2 ± 0.2% (w/w) for soil sampling. Soil and earthworms were sampled in the 0–15 cm topsoil in the center of the inter-row. There were one soil and one earthworm sample per subplot. Each soil sample consisted of a composite of four subsamples, one per inter-row, taken with a gouge auger. Soil used to measure bulk density was sampled according to the cylinder method. Soil samples were sieved at 1 cm before biological analyses and at 2 mm before physical and chemical analyses. To sample earthworms, a monolith of soil of 45 cm × 45 cm on 15 cm depth was extracted per subplot. Earthworms were sampled by the hand-sorting method and placed in alcohol solution at 75%, then transferred into a 4% formaldehyde solution to be stored.

2.3. Physical and chemical analyses

Soil used to measure bulk density was dried at 105 °C for 1 week and weighed rapidly thereafter (ISO 11272, 1998). Total organic carbon (TOC) and total nitrogen (N) contents were measured by dry combustion according to the ISO 10694 (1995) norm for TOC and the ISO 13878 (1998) norm for N. The effective cation exchange capacity (CEC) was determined according to the cobaltihexamine chloride method (ISO 23470, 2007). A water extract (soil:extractant ratio 1:10 and 2 h of contact) was used to determine the contents of available phosphorus (P), potassium (K) and copper (Cu). The solution was centrifuged (2000 × g during 20 min at 20 °C) and filtered at 0.2 μm. The P content was determined by the green malachite method (Ohno and Zibilske, 1991). The K and Cu contents were measured by flame atomic absorption spectrometry (Varian A600).

2.4. Biological analyses

Soil microbial biomass carbon (MB) was determined following the fumigation-extraction method (Wu et al., 1990). The organic carbon from fumigated and non-fumigated soils was measured with a total organic carbon analyzer TOC-V CSH (Shimadzu). Nematodes were extracted from 200 g of wet soil using the Oostenbrink

Table 1
Agricultural practices in conventional farming and organic farming.

	Soil management		Fertilization (N–P–K)	Vine phytosanitary protection	Tractor frequency per year (year^{-1})
	Rows	Inter-rows			
Conventional	Chemical weeding (glyphosate, 700 g ha^{-1}, 1 year^{-1})	Tillage with tined tools (15 cm depth, 2 year^{-1})	Mineral (10–10–20, 200 kg ha^{-1}, 1 year^{-1})	Synthesis and natural (6 treatments year^{-1})	14
Organic	Tillage (10 cm of depth, 1 year^{-1})	Mouldboard ploughing (25 cm depth, 4 year^{-1})	Compost (90% of OM) (9–5–0, 500 kg ha^{-1}, 1 year^{-1})	Natural (8 treatments year^{-1})	18

elutriation technique, complemented with sieving and cottonwood extraction (ISO 23611-4, 2007). Nematodes were fixed in a 4% formaldehyde solution and a representative sub-sample was mounted on glass slides for identification at high magnification (×400). An average of 150 nematodes per sample was identified to family level and grouped into 5 trophic groups: plant-feeders (PF), bacterial-feeders (Ba), fungal-feeders (Fu), omnivores (Om) and predators (Pr). Earthworms were gently dried before being weighed and counted. Adult and juvenile earthworms were distinguished and distributed into 2 ecological categories: endogeics and anecics.

2.5. Statistical analyses

An univariate approach using generalized and linear mixed models for hierarchical data (Pinherio and Bates, 2000; Bolker et al., 2009) was used to study the differences between treatments for each observed variable. Prior to the analysis and when necessary, the distribution of observed variables were adjusted to Gaussian distribution using proper transformations (square root or logarithmic). The variables describing density data belonging to nematode trophic groups and earthworm ecological categories were not normalized because they rather followed Poisson distribution. The functions *lmer* and *glmer* from the library lme4 of the R 2.11.1 software (R Development Core Team, 2011) were used to compute mixed models from variables having, respectively, Gaussian and Poisson distributions. The multiple comparisons of mean among treatments were then tested using Markov Chain Monte Carlo samples.

Based on the results of the univariate approach, a linear discriminant analysis was conducted to discriminate observations among the 4 treatments (Conventional, Organic7, Organic11 and Organic17). In order to do this, this multivariate analysis computed the best discriminant functions to differentiate objects among treatments, while minimizing variability within a treatment (Legendre and Legendre, 1998). Bulk density, total organic carbon (TOC) and total nitrogen (N) contents, soil microbial biomass carbon (MB), available phosphorus (P), potassium (K) and copper (Cu) contents, effective CEC (CEC), plant-feeding (PF), bacterial-feeding (Ba), fungal-feeding (Fu), combined omnivore and predator (Om + Pr) nematode densities and endogeic earthworm density and biomass were the variables integrated in the discriminant analysis. This analysis was performed using the XL-Stat software for Windows®. The results were presented in the form of correlations circle of variables, distribution of the 96 observations along the two discriminant axes and confusion matrix comparing *a priori* (real) and *a posteriori* (calculated) classification of observations using the cross-validation technique.

3. Results

3.1. Physical and chemical parameters

The Organic plots tended to have a higher bulk density than the Conventional plots but only bulk density of Organic11 was significantly higher than that of Conventional plots (Table 2). The TOC content significantly increased from Conventional to Organic17 (+32%). A marked rise was measured between Organic7 and Organic11 (+15%). Concerning the total N content, 2 different groups were identified. Conventional and Organic7 had significantly lower N content than Organic11 and Organic17. A steep decrease in available P content was measured between Conventional and Organic7 (−58%) but, thereafter, a continuous increase of available P content was noticed between Organic7 to Organic11 (+43%) and Organic11 to Organic17 (+65%). The available K content significantly increased from Conventional to Organic17 (+81%). No significant difference was measured for available Cu content between treatments but one should note that the highest values were measured in Organic plots. The highest values of effective CEC were measured for Organic7 and Organic11. In these treatments, effective CEC was significantly higher (+27%) in comparison with Conventional and Organic17.

3.2. Biological parameters

Organic11 and Organic 17 had significantly higher soil MB than Conventional and Organic7 (+34%) (Table 3). The lowest total nematode density was measured for Conventional plots. The total nematode density was significantly higher in Organic7 (+45%) and in Organic17 (+79%) than in Conventional plots. The plant-feeder density was significantly higher in Organic7 (+126%) and in Organic17 (+187%) than in Conventional plots. Plant-feeding nematodes were dominated by facultative plant-feeders with Tylenchidae family (data not shown). Among obligate plant-

Table 2
Physical and chemical parameters: bulk density, total organic carbon (TOC), total nitrogen (N), available phosphorus (P), potassium (K) and copper (Cu) contents and effective cation exchange capacity (CEC) in conventional farming (Conventional) and organic farming for 7 years (Organic7), 11 years (Organic11) and 17 years (Organic17).

	Bulk density (g cm^{-3})*	TOC (mg g^{-1})*	N (mg g^{-1})*	P (mg kg^{-1})*	K (mg kg^{-1})¥	Cu (mg kg^{-1})ns	CEC (cmol^{+} kg^{-1})*
Conventional	1.21 ± 0.03 b	10.2 ± 0.3 c	1.00 ± 0.02 b	1.06 ± 0.12 a	27 ± 3 c	0.22 ± 0.03 a	16.3 ± 0.5 b
Organic7	1.31 ± 0.01 ab	10.8 ± 0.4 bc	1.01 ± 0.03 b	0.45 ± 0.04 b	30 ± 3 bc	0.35 ± 0.07 a	19.9 ± 0.7 a
Organic11	1.41 ± 0.02 a	12.4 ± 0.4 ab	1.25 ± 0.05 a	0.64 ± 0.04 ab	45 ± 5 ab	0.34 ± 0.06 a	20.7 ± 0.8 a
Organic17	1.29 ± 0.01 ab	13.5 ± 0.5 a	1.36 ± 0.04 a	1.05 ± 0.06 a	49 ± 5 a	0.39 ± 0.07 a	15.7 ± 0.4 b

Means ± standards errors are presented. Means differing significantly are denoted with different lowercase letters; ns, not significant.

* Threshold significant at 95%.

¥ Threshold significant at 90%.

Table 3
Soil microbial biomass (MB) and nematode trophic group density in conventional farming (Conventional) and organic farming for 7 years (Organic7), 11 years (Organic11) and 17 years (Organic17).

	Microorganisms	Nematofauna (ind. (100 g dry soil)$^{-1}$)				
	MB ($\mu g\,C\,g^{-1}$)[¥]	Plant-feeders[*]	Bacterial-feeders[ns]	Fungal-feeders[¥]	Omnivores–predators[¥]	Total[¥]
Conventional	77 ± 7 b	210 ± 17 c	402 ± 29 a	212 ± 15 b	112 ± 12 b	936 ± 49 c
Organic7	73 ± 3 b	474 ± 62 ab	422 ± 36 a	303 ± 38 a	156 ± 20 a	1355 ± 115 ab
Organic11	100 ± 5 a	403 ± 69 b	440 ± 55 a	303 ± 37 a	101 ± 17 b	1248 ± 137 bc
Organic17	101 ± 4 a	603 ± 62 a	541 ± 60 a	417 ± 51 a	113 ± 13 b	1672 ± 143 a

Means ± standards errors are presented. Means differing significantly are denoted with different lowercase letters; ns, not significant.

[*] Threshold significant at 95%.

[¥] Threshold significant at 90%.

feeders, *Paratylenchus* sp. and *Tylenchorynchus* sp. dominated and almost no *Xiphinema* sp. were found whatever the treatment, just 7.5 and 12.1 ind. (100 g dry soil)$^{-1}$ for 2 samples (data not shown). No significant difference was measured for bacterial-feeder densities between treatments. However, the lowest density was observed for Conventional plots. The fungal-feeder density increased from Conventional to Organic17. It was significantly higher in Organic7 (+43%) and in Organic17 (+97%) than in Conventional plots. The combined density of omnivores and predators was significantly higher (+44%) in Organic7 than in other treatments.

There were many samples without earthworms in all the treatments, and especially in the Organic plots (Table 4). The total earthworm density was significantly higher in Conventional than in Organic plots (−44% between Conventional and Organic7 and −55% between Conventional and Organic17). Endogeics were the most represented (more than 85% of total density) compared to the anecics in both Conventional and Organic plots. The anecic density was low whatever the treatment and not significantly different between treatments. The highest biomass of earthworms was observed in the Conventional plots. Contrary to the density, the biomass was mainly represented by the anecics.

3.3. Discriminant analysis

The discriminant analysis (Fig. 1 and Table 5) was significant at $\alpha = 0.001$ according to the Wilks test and each discriminant axis explained a significant portion of the overall variance of the database ($p < 0.01$). The first two discriminant functions explained 93% of the dataset variability (Fig. 1A). The first axis explained 68% of the dataset variability and was mainly defined by total N and TOC contents, plant-feeding nematode density, available K content and fungal-feeding nematode density. The axis 2 contributed 25% of the dataset variability and was correlated to effective CEC and available P content. The distribution of observations along the 2 axes showed a clear discrimination of the 4 treatments (Fig. 1B). The first axis clearly discriminated the 4 treatments and therefore, a gradient starting from Conventional to Organic17 was found along this axis. On the other hand, the second axis was less discriminant as it only discriminated Conventional and Organic17 from Organic7 and Organic11. Some overlaps were observed between treatments and were associated to the variability of observations in a given treatment. The confusion matrix (Table 5) compared the classification of plots predicted by the model of discriminant analysis and the real classification constituted from experimental plot design. The confusion matrix showed that the discriminant analysis successfully classified 79% of the 96 observations. The *a posteriori* classification of Conventional and Organic17 plots were respectively correct at a rate of 88 and 90% whereas, Organic7 and Organic11 plots were correctly classified, respectively at a rate of 75% and 55%.

4. Discussion

4.1. Soil quality indicators to study the transition to organic farming

This study was conducted on commercial vineyards. Fourteen plots which have been organically managed for 7, 11 and 17 years were compared to 10 conventionally managed plots. We analysed the long-term effects of organic management on vineyard soil quality determined by physical, chemical and also biological indicators. Measurements on grape production were not included to define soil quality because the diversity of plant material would not have given reliable data to compare the different treatments. As reviewed by Bastida et al. (2008), the multiparametric indices are a promising tool to objectively describe the changes of soil quality but the weighting of different functions is subjective and does not depend on statistical (objective) method. In this study, our statistical approach was different because we used a combination of statistical tools that clearly distinguish the four treatments which organized themselves along the first axis, defining a gradient starting from Conventional to Organic17. Indeed, no significant difference appeared before 11 years of organic farming for total organic carbon (TOC), total nitrogen (N) and available potassium (K) contents, soil microbial biomass (MB) and fungal-feeding nematode density (Fu). However, other soil indicators, such as available P content rapidly decreased after conversion. Some authors, such as Martin et al. (2007), explained such a trend as an exhaustion of available P pools built up from successive mineral P fertilizers. Afterwards, we observed an increase of available P content as reported by Garcia-Ruiz et al. (2009) and Liu et al. (2007) on differ-

Table 4
Proportion of samples without earthworm, density and biomass of endogeic and anecic earthworms in conventional farming (Conventional) and organic farming for 7 years (Organic7), 11 years (Organic11) and 17 years (Organic17).

	Samples without earthworm (%)	Density (ind m^{-2})			Biomass (g m^{-2})		
		Endogeics[¥]	Anecics[ns]	Total[¥]	Endogeics[ns]	Anecics[ns]	Total[ns]
Conventional	45	12.5 ± 2.6 a	1.1 ± 0.5 a	13.6 ± 2.6 a	4.5 ± 1.1 a	15.5 ± 8.9 a	19.9 ± 9.1 a
Organic7	75	6.9 ± 5.5 b	0.7 ± 0.7 a	7.6 ± 5.5 b	0.6 ± 0.6 a	1.1 ± 1.1 a	1.8 ± 1.3 a
Organic11	75	6.1 ± 4.0 b	0.6 ± 0.6 a	6.7 ± 4.0 b	1.0 ± 0.9 a	8.1 ± 8.1 a	9.0 ± 8.1 a
Organic17	60	4.4 ± 2.0 b	1.7 ± 0.9 a	6.1 ± 2.1 b	0.3 ± 0.1 a	11.3 ± 7.0 a	11.7 ± 7.0 a

Means ± standards errors are presented. Means differing significantly are denoted with different lowercase letters; ns, not significant.

[¥] Threshold significant at 90%.

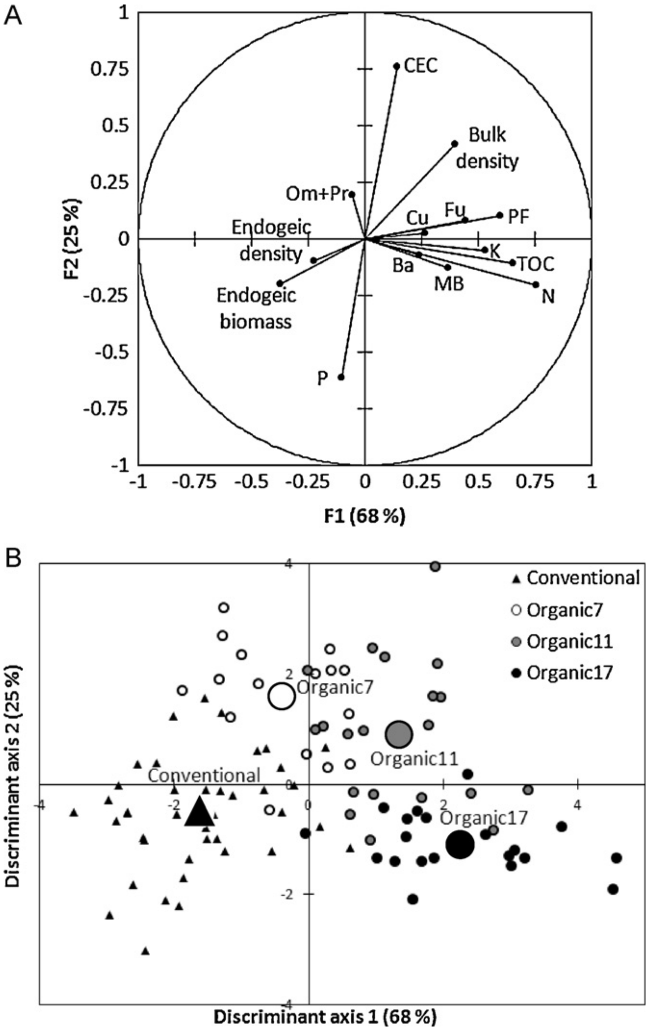

Fig. 1. Discriminant analysis performed on physical, chemical and biological parameters for conventional farming (Conventional) and organic farming for 7 years (Organic7), 11 years (Organic11) and 17 years (Organic17). (A) Correlations circle of variables: bulk density, total organic carbon (TOC), total nitrogen (N), available phosphorus (P), potassium (K) and copper (Cu) contents, effective cation exchange capacity (CEC), soil microbial biomass (MB), density of plant-feeding (PF), bacterial-feeding (Ba), fungal-feeding (Fu), omnivore and predator (Om + Pr) nematodes and density and biomass of endogeic earthworms. (B) Distribution of the 96 observations and of the centroids (prominent symbols) of each treatment along the 2 discriminant axes.

ent crops. Conversely, available K content increased progressively from Conventional to Organic17 whereas Gosling and Shepherd (2005) observed K was significantly higher in the conventional fields than in the low fertility organic fields. One should note that the quantity of compost applied was too low to explain the observed trends for P and K (25 kg P ha^{-1} year^{-1} and no K, Table 1). Previous studies have shown that microorganisms release organic acids which can increase the availability of P (Arcand and Schneider, 2006) and K (Basak and Biswas, 2009). Phosphatases excreted by microorganisms, and more particularly fungi, have also the ability to mineralize organic P (Rodriguez and Fraga, 1999). Thus, the increase of microbial biomass after organic conversion and activities of some microorganisms could explain why after 7 years of organic farming, the available P and K contents increased. The total nematode density, especially plant-feeders (PF) and total earthworm density, especially endogeic density responded more sharply and quickly to the organic conversion. Garcia-Ruiz et al. (2009) did the same observation for the plant-feeder density in one site of olive groves after 7–8 years of organic practices. In our case, plant-feeders that increased in density were mainly the facultative plant-feeders Tylenchidae and phytoparasitics, *Tylenchorynchus* sp. *Xiphinema* sp., grapevine fanleaf virus vector (GFLV) were almost absent in each treatment. However, it is known that the highest density of *Xiphinema* sp. occurred at 40–110 cm depth and yet we sampled it in the 0–15 cm topsoil (Villate et al., 2008). For earthworm density, Scullion et al. (2007) observed some positive effects after 10 years of organic farming on grass-arable rotations. Gradual changes of soil properties in converting plots into organic farming have been measured by several authors and discussed by Martini et al. (2004). The other studied indicators did not present any change and should be considered as not sensitive enough to monitor the conversion to organic viticulture in these pedo-climatic conditions. So, organic matter, available P and K, microbial biomass, nematodes and earthworms could be considered as a basis to a guideline to best transition strategies in vineyards.

4.2. *Effects of organic farming on soil organisms*

In organic farming, plant nutrition is based on the mineralization of organic matter by soil organisms. Several authors reported negative effects of Cu on soil organisms (Wightwick et al., 2010). We observed, as Beni and Rossi (2009), trends of higher available Cu contents in Organic treatments than in Conventional ones. As a matter of facts, Cu salts are the only efficient fungicides against downy mildew, allowed in organic farming. Furthermore, Brun et al. (2001) showed Cu accumulates in the upper layers of soils. However in our study, we did not observe significant increase of available Cu after organic conversion. This might be related to the method that we used for measuring Cu availability. Michaud et al. (2007) have shown that in calcareous soils contaminated by Cu fungicides, water-extractable Cu varied little, compared to total soil Cu, or EDTA-extractable Cu.

Generally, grass cover and applications of organic matter have positive effects on earthworms in vineyards (Peres et al., 1998; Paoletti, 1999; Eisenhauer et al., 2009). However, we measured the lowest density and biomass of earthworms in Organic plots. The density of anecics was very low in all treatments but it was certainly underestimated. Actually, the hand-sorting method without prior application of an expellant (such as mustard or formaldehyde solution) did not allow to sample larger earthworms, such as anecics, which can rapidly escape into deeper soil layers (Pelosi et al., 2009). However, the hand-sorting is appropriate for endogeic and their density strongly decreased between Conventional and

Table 5
Confusion matrix comparing *a priori* (real) and *a posteriori* (calculated) classification of observations using the cross-validation technique.

	A posteriori classification				Correct classifications (%)
	Conventional	Organic7	Organic11	Organic17	
A priori classification					
Conventional	35	3	2	0	88
Organic7	3	12	1	0	75
Organic11	0	3	11	6	55
Organic17	1	0	1	18	90

Organic7. The tillage which was more frequent to control weeds under organic management could also explain these observations. Several authors highlighted negative effects of tillage on earthworm density and biomass (Emmerling, 2001; Paoletti, 1999 on different crops). Furthermore, with the organic conversion, the shallow tillage was replaced by a deeper mouldboard ploughing. Short-term studies (3 and 6 years) of Metzke et al. (2007) and Peigné et al. (2009) on different tillage systems under organic annual crop farming did not observe higher earthworm density or biomass under shallow tillage in comparison with mouldboard ploughing. However, we could hypothesize that mouldboard ploughing has long-term negative effect on earthworms. Villenave et al. (2009) demonstrated that tillage also tends to disturb omnivore and predator nematodes. In the same way, we did not measure any modification of the densities of these nematodes with organic farming and their low densities in every plot indicated a simple and short soil micro-food web in all these vineyards (Ferris et al., 2001). Soil compaction, as evaluated by the measure of bulk density, was higher in Organic plots. This was the consequence of the increase of the traffic for tillage and phytosanitary treatments in organic management. Bouwman and Arts (2000) observed a decrease of free-living nematode density in a heavily compacted soil compared with a slightly compacted soil. They explained that nematodes reacted negatively to the decreased pore space habitats due to soil compaction. In our case, bacterial and fungal-feeding nematode density was globally higher in Organic plots despite an increased bulk density. Hansen (1996) and Hansen and Engelstad (1999) showed that soil compaction had negative effects on earthworms. So, soil compaction could explain, with ploughing, the decrease of earthworm density that we observed in organic viticulture. More globally, agricultural practices in organic conversion cause some damage on soil organisms.

4.3. Organic matter, microbial biomass and nematodes

The soil organic matter plays essential roles in soil functioning and it can be considered as the keystone of soil management under organic farming. Under Organic plots, we measured higher TOC contents. Many authors observed the same positive effect of organic farming as Briar et al. (2007) on different annual crops and Vestberg et al. (2009) on strawberry crops. The sole application of compost (261 kg of organic carbon ha^{-1} $year^{-1}$) would lead to a content of 11.2 mg of TOC g^{-1} of soil after 17 years of organic farming whereas we measured it at 13.5 mg g^{-1}. Thus, the more abundant grass cover in Organic treatments contributed certainly in the increase of TOC contents and microbial biomass as observed by Potthoff et al. (2006). Organic matter constitutes a source of nutrients for microorganisms (Calbrix et al., 2007) which increased after the conversion into organic farming and root-exudates are known too to stimulate microbial growth (Bouwman and Arts, 2000; Whitelaw-Weckert et al., 2007). In the same way, Rahman et al. (2009) showed positive effects of grass cover on the density of plant-feeding and microbivorous nematodes which agreed with our results. An increase of plant-feeding nematodes intensifies root C fluxes activating microbial biomass from the rhizosphere and microbivorous nematodes stimulate microorganisms (Denton et al., 1999). Conversely, as there were more microorganisms in Organic plots, we consistently found more fungal-feeding nematode density in organic plots, which is in line with Ferris et al. (1996), Villenave et al. (2004) and Villenave et al. (2010). However, only an increasing trend was observed for bacterial-feeding nematode density in Organic plots. The nematodes are good indicators of soil decomposition pathway (Ferris et al., 2004). The bacterial-feeders/fungal-feeders ratio decreased with organic farming, as observed by Mc Sorley and Frederick (1999). So, the decomposition pathway becomes dominated by fungi under Organic plots in comparison with Conventional plots. The increase of fungi after organic conversion was also observed by different authors as Gryndler et al. (2006) for arbuscular mycorrhizal fungi. Thus, the application of compost and the presence of a grass cover on Organic plots led to increase soil organic matter, microbial biomass and nematode density, especially plant-feeders and fungal-feeders whereas ratio bacteria/fungi decreased.

5. Conclusion

Through our experimental design, we studied long-term effects of different changes of agricultural practices inherent to organic viticulture on soil quality. In this study, we have demonstrated that a transition period of 7–11 years, depending on the considered indicator, was needed to clearly separate Conventional and Organic farming practices in Southern French vineyards. Apart from classical sensitive indicators used to study organic transition like organic matter content, soil microbial biomass, or bulk density, the easy-to-use chemical available P and K contents should also be considered as sensitive indicators. Moreover, our results address the important question of P and K mining with organic practices during transition period. However afterwards, the increase of soil organic matter and related biological activities could partly counteract the observed decrease during the transition period. According to our results, the utilization of soil nematodes as bioindicators of soil quality shall be promoted. Indeed, the study of microbivorous provided some information about microbial biomass and bacteria/fungi ratio. As for omnivore and predator nematodes, they were reliable indicators of environmental perturbations. Despite the diversity of indicators, we have highlighted the difficulty to show the benefits of organic farming on global soil quality in this particular pedoclimatic area, and for the set of farming practices that were investigated.

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Soil pH controls the environmental availability of phosphorus: Experimental and mechanistic modelling approaches

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ABSTRACT

Inorganic P is the least mobile major nutrient in most soils and is frequently the prime limiting factor for plant growth in terrestrial ecosystems. In this study, the extraction of soil inorganic P with $CaCl_2$ (P–$CaCl_2$) and geochemical modelling were combined in order to unravel the processes controlling the environmentally available P (EAP) of a soil over a range of pH values (pH $\sim$ 4–10). Mechanistic descriptions of the adsorption of cations and anions by the soil constituents were used (1-pK Triple Plane, ion-exchange and NICA-Donnan models). These models are implemented into the geochemical code Visual MINTEQ. An additive approach was used for their application to the surface horizon of a Cambisol. The geochemical code accurately reproduced the concentration of extracted P at the different soil pH values ($R^2 = 0.9$, RMSE = 0.03 mg kg^{-1}). Model parameters were either directly found in the literature or estimated by fitting published experimental results in single mineral systems. The strong agreement between measurements and modelling results demonstrated that adsorption processes exerted a major control on the EAP of the soil over a large range of pH values. An influence of the precipitation of P-containing mineral is discounted based on thermodynamic calculations. Modelling results indicated that the variations in P–$CaCl_2$ with soil pH were controlled by the deprotonation/protonation of the surface hydroxyl groups, the distribution of P surface complexes, and the adsorption of Ca and Cl from the electrolyte background. Iron-oxides and gibbsite were found to be the major P-adsorbing soil constituents at acidic and alkaline pHs, whereas P was mainly adsorbed by clay minerals at intermediate pH values. This study demonstrates the efficacy of geochemical modelling to understand soil processes, and the applicability of mechanistic adsorption models to a 'real' soil, with its mineralogical complexity and the additional contribution of soil organic matter.

1. Introduction

Inorganic P is the least mobile major nutrient in most soils and is often the prime limiting factor for plant growth in terrestrial ecosystems (e.g. Hinsinger, 2001; Raghothama and Karthikeyan, 2005) as well as for biota in aquatic ecosystems (e.g. Smith et al., 1999). Furthermore, the concentration of P that is potentially available for plants, hereafter referred to as environmentally available P (EAP), is seldom correlated with the total concentration of P in a soil or sediment. This difference reveals that the EAP is the result of complex interactions between physical, chemical and biological processes (Murrmann and Peech, 1969; Sharpley and Tunney, 2000; Hinsinger, 2001; Lanno et al., 2004; Linge and Oldham, 2004; Marschner et al., 2005). Moreover, these processes are also influenced by soil solution variables such as ionic strength, concentrations of anions or cations, pH, etc. Change in soil conditions is particularly large and rapid in the bio-influenced zone near the root, the rhizosphere, where the conditions, e.g. pH, can be quite different from those of the bulk environment, thus resulting in altered environmental availability (Harmsen et al., 2005). When applying this concept to P in soil–plant systems, the rhizosphere can indeed show large pH changes relative to the bulk soil, with a potentially large impact on the EAP (Gahoonia et al., 1992; Bertrand et al., 1999; Hinsinger et al., 2003). However, it is particularly difficult to predict to what extent and even in what direction (decrease or increase) the EAP would change in response to a variation of soil pH (Hinsinger, 2001). Hinsinger et al. (2009) considered the effects of pH variations on the EAP as the major limitation of most advanced plant nutrition models at predicting the uptake of P by plants when the EAP is low (e.g. the model of Mollier et al., 2008).

In practice, the EAP is expressed as the concentration of inorganic P that is chemically extracted from a soil sample with a given extracting agent (e.g. Anshumali and Ramanathan, 2007). In Europe, up to 17 extraction methods have been used to assess the EAP, which suggests that none is satisfactory (Harmsen et al., 2005). The extraction of P from a soil sample by $CaCl_2$ was first

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proposed by Houba et al. (1990). Since then, this method has been increasingly used for environmental applications (McDowell and Condron, 2000; McDowell and Sharpley, 2001; Maguire and Sims, 2002; McDowell et al., 2003; Harmsen et al., 2005; Rhoton and Bighman, 2005; Hartz and Johnstone, 2006). The concentration of P extracted by $CaCl_2$ (hereafter referred to as P–$CaCl_2$) is widely used as a risk assessment indicator of P-losses from soils (McDowell and Sharpley, 2001). In addition, the positive correlation between P–$CaCl_2$ and the concentration of P extracted with the widely used Olsen method (common soil testing procedure in agricultural soils) further validates its potential use as an indicator of the EAP for assessing P available to plants (Ehlert et al., 2003). The extraction of P by $CaCl_2$ has thus provided satisfactory results in many environmental applications. Nevertheless, the hierarchy of the processes and mechanisms controlling the EAP concentrations in different soils still remain largely unknown (Hesketh and Brookes, 2000; Neyroud and Lischer, 2003). As suggested by Van Erp (2002), the mechanisms responsible for concentration variations of ions extracted by $CaCl_2$ can be better understood with numerical models, and especially by using mechanistic approaches.

Several geochemical models based on a mechanistic description of adsorption processes have been developed over the last decade (e.g. Kinniburgh et al., 1999; Hiemstra and Van Riemsdijk, 1998). These tools have notably been used to simulate the influence of pH, cations and anions on the concentrations of P adsorbed at equilibrium onto various solid phases (e.g. Frau et al., 2008). However, these studies concerned simple systems, made of a single mineral or a mixture of two minerals. To the best of the authors' knowledge, only one previous study has been devoted to P adsorption in complex systems such as soils (Gustafsson, 2001). This may be due to the lack of equilibrium constants relevant to the range of minerals and adsorbed cations and anions that can affect P adsorption in soils.

The main objective of this study was to investigate the relevance of mechanistic adsorption models for simulating the EAP in a soil. For this purpose, generic model parameters were retrieved from the literature. Geochemical modelling was also used in an attempt to unravel the geochemical processes that control the EAP in a soil, as appreciated through P–$CaCl_2$. Measurements and modelling were both performed at various soil pH values, in order to cover a range of environmental conditions and thereby to better challenge the modelling approach of the EAP.

Table 1

Chemical properties of the studied soil (0–20 cm depth), a Chromic Cambisol sampled at Cazevieille, France. The names of the extractant are indicated in brackets.

pH ($CaCl_2$)	7.2
Organic C	31 g kg^{-1}
Total Inorganic C	1.48 g kg^{-1}
Total P	950 mg kg^{-1}
CEC (cobalthexamine)	25 cmol(+) kg^{-1}
Ca (cobalthexamine)	22.3 cmol(+) kg^{-1}
Mg (cobalthexamine)	0.977 cmol(+) kg^{-1}
Na (cobalthexamine)	0.121 cmol(+) kg^{-1}
K (cobalthexamine)	0.213 cmol(+) kg^{-1}
Fe (cobalthexamine)	0.0328 cmol(+) kg^{-1}
Al (cobalthexamine)	0.0416 cmol(+) kg^{-1}
Fe (ammonium oxalate)	1.63 g kg^{-1}
Al (ammonium oxalate)	4.58 g kg^{-1}
Si (ammonium oxalate)	1.34 g kg^{-1}
P (ammonium oxalate)	125 mg kg^{-1}
Fe (EDTA)	9.25 mg kg^{-1}
Al ($CuCl_2$)	3.4 mg kg^{-1}
Fe (CDB)	32.5 g kg^{-1}
Al (CDB)	5 g kg^{-1}
Si (CDB)	2.2 g kg^{-1}
Total Fe (in <2 μm fraction)	62 g kg^{-1}
Total Al (in <2 μm fraction)	115 g kg^{-1}
Total Si (in <2 μm fraction)	176 g kg^{-1}

2. Material and methods

2.1. Soil properties and experimental data

A topsoil (0–20 cm depth) sampled at Cazevieille (Hérault, France) (43°46′N, 3°47′E) was studied. The site corresponds to xerotypic shrubland developed in a humid Mediterranean climate. The soil is classified as a Chromic Cambisol (FAO-UNESCO, 1989), with a silty clay texture.

The soil sample was air dried and sieved to 2 mm prior to chemical analysis. Measured chemical properties of the soil are provided in Table 1. The total concentration of P in the soil material, which includes occluded P, mineral P, adsorbed P etc., was estimated by digestion with H_2SO_4 and $HClO_4$ at a volume:volume ratio of 20:1 (e.g. Li et al., 2008). The total adsorbed P concentration was estimated by an ammonium oxalate extraction (Guo and Yost, 1999). The cation exchange capacity (CEC) and the concentrations of the cations adsorbed by the soil material were determined by extraction with cobalthexamine chloride (Lofts et al., 2001).

The mineralogy of the soil was determined using XRD analysis and standard chemical extractions. A Rigaku diffractometer equipped with a Co X-ray source and a CGR monochromator was used. In addition to the primary minerals (quartz and K-feldspar), XRD analysis showed the presence of illite, kaolinite and Fe-oxides. The occurrence of Al-oxides was derived from chemical extractions. To assess the amounts of kaolinite and illite in the soil material, the total concentrations of Si, Al, Fe and K measured in the clay-size fraction were assigned to the chemical composition of kaolinite and illite, according to the following formulas: $[Si_4O_{10}][Al_4(OH)_8]$ and $[(Si_{6.7}Al_{1.3})O_{20}][(Al_{1.95}Fe^{3+}_{1.95})(Fe^{2+}_{0.05}Mg_{0.05})(OH)_4]K_{1.3}$, respectively (Fanning et al., 1989). Calculation showed that the clay concentration represented 70% of the clay-size fraction. Within this proportion, it was further calculated that the respective abundance of illite and kaolinite was fairly close to each other, i.e. 54% and 46%, respectively. To estimate the concentrations of Al- and Fe-oxides, extractions by ammonium oxalate and citrate–dithionite–bicarbonate (CDB) were used (Mehra and Jackson, 1960; de Endredy, 1963). The concentration of Fe extracted by ammonium oxalate was assumed to be ferrihydrite, which is a poorly-crystalline form of Fe-oxide (e.g. Gustafsson, 2001). The amount of crystalline Fe-oxides was estimated by subtracting the concentration obtained by ammonium oxalate from that obtained by CDB extraction. It was assumed that the crystalline Fe-oxide detected in this soil was goethite (Mehra and Jackson, 1960; Carreira and Lajtha, 1997). Regarding Al-oxides, their concentrations were determined from the amount of Al extracted by CBD extraction (Gustafsson, 2001). In this study, Al-oxides were assumed to be gibbsite. Not surprisingly for a chromic Cambisol, the ratio between Si and Al concentrations extracted by ammonium oxalate indicated the absence of allophane and imogolite type material (e.g. Gérard et al., 2002, 2003). The concentration of Al extracted by $CuCl_2$ (Juo and Kamprath, 1979) showed little organically-bound Al, which is quite consistent with the neutral pH of the soil.

Air-dried and sieved (2 mm) soil subsamples were acidified or alkalinised by adding 5–200 mmol of either H_2SO_4 or KOH per kg of air-dry soil. After this, the water content of each sample was adjusted to 0.58 g g^{-1} of soil (W/R = 0.58) and samples were incubated at 25 °C over 84 h. Extractions of inorganic P with $CaCl_2$ (0.01 M) at a ratio of 1:5 were performed according to the procedure proposed by Houba et al. (1990). Each sample was shaken for 30 min and left 15 min to settle down. Supernatants were filtered through 0.2 μm cellulose acetate membrane filter (VWR International). The concentration of P in the extracts was measured with the malachite green method (Ohno and Zibilske, 1991; Huang and Zhang, 2006). The soil pH was measured in $CaCl_2$ supernatants using a Metrohm-744 pH meter.

2.2. Geochemical modelling

The additive approach was used to simulate the EAP of the soil samples set at different pH values. This approach considers that the properties of the overall soil can be described as the sum of the individual properties of its constituents (Davis et al., 1998; Gustafsson, 2001). In order to simulate the adsorption behaviour of the different soil constituents, three adsorption models were used: (i) the 1-pK Triple Plane Model (TPM) for the adsorption of anions and cations by minerals (Hiemstra and Van Riemsdijk, 1998), (ii) ion-exchange for cation adsorption on permanent negatively charged sites of clays and (iii) NICA-Donnan for the adsorption of cations by the soil organic matter.

The 1-pK TPM includes a multi-site complexation model (Hiemstra et al., 1989) and a charge distribution model based on the Pauling concept (Hiemstra and Van Riemsdijk, 1996). A single equilibrium constant (K_1) corresponding to the point of zero charge (PZC) of the mineral surfaces is used to describe protonation/deprotonation reactions. These reactions occur at hydroxyl sites bound to Al or Fe atoms (noted ≡S) of the mineral structure, as represented by the following reaction:

$$\equiv SOH_{(s)}^{-1/2} + H^+_{(aq)} \leftrightarrow \equiv SOH_{2(s)}^{+1/2} \tag{1}$$

with the equilibrium constant K_1 expressed as:

$$K_1 = \frac{\{\equiv SOH_2^{+1/2}\}\{H_2O\}}{\{H^+\}\{\equiv SOH^{-1/2}\}} \tag{2}$$

where the parentheses represent the activity of the corresponding chemical species. The concentration of surface hydroxyl sites is a function of the concentration of the mineral phase and of its specific surface area and site density.

The adsorption of P as well as the other ions is also described by the stoichiometry of the reactions and their equilibrium constants (K_2). For example, for P adsorbed as an innersphere complex the reaction can be written as:

$$\equiv SOH_{(s)}^{-1/2} + H^+_{(aq)} + PO_{4(aq)}^{3-} \leftrightarrow \equiv SOPO_{3(s)}^{2.5-} + H_2O \tag{3}$$

with the equilibrium constant K_2 given by:

$$K_2 = \frac{\{\equiv SOPO_3^{2.5-}\}\{H_2O\}}{\{\equiv SOH^{-1/2}\}\{H^+\}\{PO_4^{3-}\}} \tag{4}$$

According to the TPM, electrical charges of the adsorbed ions are distributed in the double-diffuse layer represented by three electrostatic planes (Fig. 1). The first plane (0-plane) corresponds to the mineral surface. The 1-plane separates the inner- and outersphere of the Stern layer. The last electrostatic plane (2-plane) separates the Stern layer from the diffuse layer (Hiemstra and Van Riemsdijk, 1996). This formalism distinguishes innersphere surface complexes from outersphere surface complexes, also corresponding to specific and non-specific adsorption processes, respectively. Ligands are shared with the surface sites when innersphere complexes are formed while outersphere complex formation involves electrostatic interactions between the adsorbed ion and the surface sites. The distribution of electrical charges added by adsorbed ions is positioned in the electrostatic planes and taken into account by means of the Boltzmann accumulation factors. These factors are defined as (Hiemstra and Van Riemsdijk, 1996):

$$P_0 = e^{-(F\psi_0/RT)} \quad P_1 = e^{-(F\psi_1/RT)} \quad P_2 = e^{-(F\psi_2/RT)} \tag{5}$$

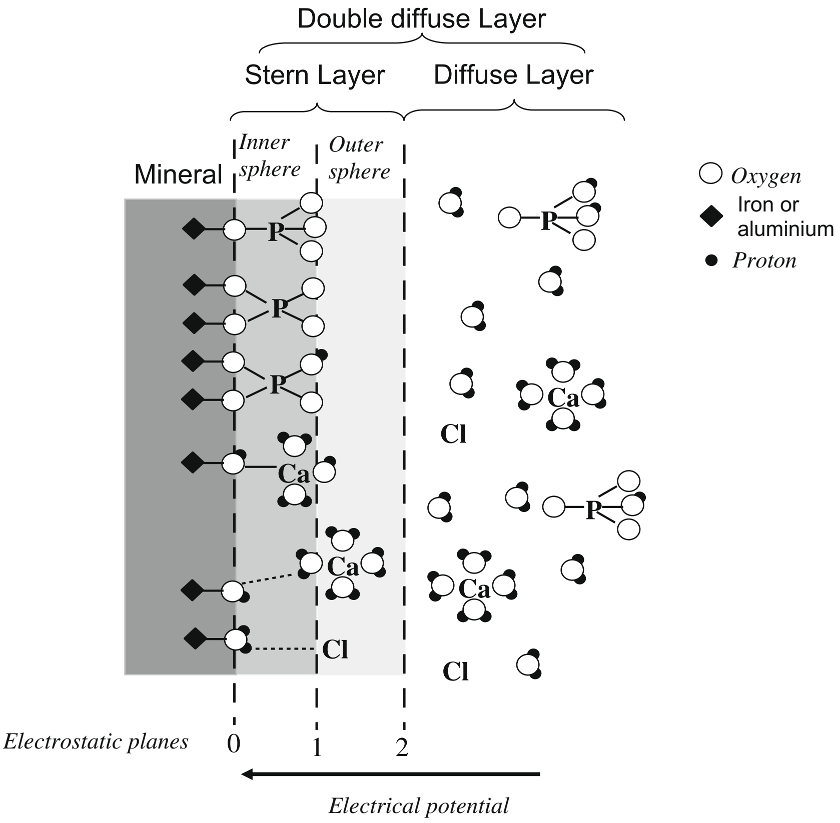

Fig. 1. Representation of the diffuse double layer at a mineral surface according to the Triple Plane Model (TPM). Three electrostatic planes separate innersphere complexes and outersphere complexes, which share ligands with the surface sites and are electrostatically bound with reactive surface hydroxyl sites, respectively.

with:

$$\sigma_0 = C_1(\psi_0 - \psi_1) \quad \sigma_1 = C_1(\psi_1 - \psi_0) + C_2(\psi_1 - \psi_2)$$
$$\sigma_2 = C_2(\psi_1 - \psi_2) \tag{6}$$

where the subscripts 0, 1 and 2 stand for the 0-, 1- and 2-plane of the diffuse layer, respectively, F is the Faraday constant (96485.34 C), R is the gas constant (8.3144 J mol^{-1} K^{-1}), T stands for the absolute temperature (K), σ and ψ correspond to the electrostatic potential (V) and the electrical charge (C m^{-2}) of each plane, respectively. The parameters C_1 and C_2 (F m^{-2}) denote the molecular capacitances of inner and outer Stern layers, respectively.

Two types of adsorption sites are present on kaolinite and illite (Lackovic et al., 2003; Gu and Evans, 2007, 2008): (i) the edge sites (noted $\equiv$S), where the adsorption of ions is modelled with the 1-pK TPM (see above) and (ii) permanent negatively charged sites associated with basal siloxane surfaces (noted $\equiv X^-$). The adsorption of cations on permanent negatively charged sites can be described by the stoichiometry of the reactions and their equilibrium constants (K_2) without electrostatic terms. For example, for the exchange of Ca^{2+} by two protons the reaction can be written as:

$$2\equiv X^- \cdot H^+ + Ca^{2+} \leftrightarrow 2\equiv X^- \cdot Ca^{2+} + 2H^+ \tag{7}$$

with the equilibrium constant K_2 given by:

$$K_2 = \frac{\{\equiv X^- \cdot H^+\}^2\{Ca^{2+}\}}{\{2\equiv X^- \cdot Ca^{2+}\}\{H^+\}^2} \tag{8}$$

The common assumption was made that the binding properties of the natural organic matter can be approximated by those of humic substances (e.g. Dudal and Gérard, 2004), and used the NICA-Donnan model to account for the adsorption of Ca by humic substances (e.g. Kinniburgh et al., 1996, 1999). In this model, cation and proton binding sites are carboxylic and phenolic groups, and the variations in the binding affinity of these two groups are continuously described with a bimodal statistic function. The total amount of component i (protons or cations) adsorbed by a fulvic or humic acid (mol kg^{-1}), $Q_{i,tot}$, can be expressed as (Kinniburgh et al., 1999):

$$Q_{i,tot} = \frac{n_{i,j}}{n_{Hj}} \sum_{j=1}^{2} Q_{j,\max} \left[\frac{K_{i,j}K_i^{el}C_i^{n_{i,j}}}{\sum_i K_{i,j}K_i^{el}C_i^{n_{i,j}}}\right] \times \left[\frac{\sum_i K_{i,j}K_i^{el}C_i^{n_{i,j}^{p_j}}}{1 + \sum_i K_{i,j}K_i^{el}C_i^{n_{i,j}^{p_j}}}\right] \tag{9}$$

where the subscript i corresponds to a cation or a proton, the subscript j corresponds to the type of binding sites (carboxylic or phenolic), $n_{i,j}$ and n_{Hj} represents the stoichiometry of the adsorption reactions for cations and protons, respectively, $n_{i,j}$ ($0 < n_{i,j} \leqslant 1$) accounts for the "non ideal" behaviour of the adsorption reaction between a cation i and a binding of the type j, $Q_{j,\max}$ stands for the maximum number of sites of type j (mol kg^{-1}), C_i refers to the dissolved concentration of the ith cation (mol l^{-1}), $K_{i,j}$ is the median equilibrium constant for the adsorption of a cation i to the binding site j, p ($0 < p \leqslant 1$) stands for the width of the distribution of each group of sites, K_i^{el} is the electrostatic contribution of the ith adsorbed cation, which is calculated with a Donnan model. In the NICA-Donnan model, the electrostatic potential surrounding humic substances are supposed constant and uniform within the domain of the gel phase and it drops to zero at the boundary between the molecule and the solution.

The numerical modelling was performed with the chemical equilibrium software Visual MINTEQ (Gustafsson, http://www.lwr.kth.se/English/OurSoftware/vminteq/). In this geochemical software, thermodynamic data for aqueous species and minerals are from the MINTEQA2 version 4.0 database (US Environmental Protection Agency, 1999).

2.3. Model parameters

The thermodynamic stability of the major Ca-phosphate minerals likely to form during the course of the experiments, especially under alkaline pH conditions, were tested (Murrmann and Peech, 1969; Carreira and Lajtha, 1997; McDowell et al., 2003). As potential secondary phases, the most common were considered: hydroxyapatite ($Ca_5(PO_4)_3OH$), octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$) and dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$). Their equilibrium constants (−44.33, −47.08 and −18.99, respectively) were those reported in McDowell and Sharpley (2001). Corresponding chemical reactions are written in terms of PO_4^{3-}, Ca^{2+}, and H$^+$ species.

The total concentration of P used as model input corresponds to the sum of the total dissolved and adsorbed P. This value was set to the value measured in the ammonium oxalate extract (see Table 1). Regarding Ca, the model input used was the sum of the Ca extracted by cobalthexamine chloride solution (see Table 1) and the Ca supplied by the $CaCl_2$ extracting solution (0.01 M). With respect to total Cl, the concentration in the $CaCl_2$ extracting solution was used.

The values of the parameters used in the 1-pK TPM are shown in Table 2a and b. The specific surface area, PZC, and site density of the five minerals considered in the study are within the range of values reported in the literature. With respect to the site density, the values used for ferrihydrite and gibbsite correspond to the density of surface hydroxyl sites sharing a bond with one Fe or Al atom (Manning and Goldberg, 1996). These types of surface hydroxyl sites are usually called single coordinated group. For goethite, surface hydroxyl sites sharing bonds with three Fe atoms, i.e. triple coordinated groups, are also taken into account (Hiemstra and Van Riemsdijk, 1996). In accordance with Wieland and Stumm (1992), the single coordinated groups were considered for the acid/base properties of illite and kaolinite. This type of binding site is localized at the edges of the clay minerals. As mentioned before, clays minerals develop two types of adsorption sites. It was assumed that the second type, corresponding to permanent negatively charged sites, represented 16% and 35% of the edge-sites concentrations for kaolinite and illite, respectively (Lackovic et al., 2003; Gu and Evans, 2008).

The inner capacitance (C_1) and the outer capacitance (C_2) used for goethite were obtained from Hiemstra and Van Riemsdijk (2006). The same values were used for ferrihydrite (Gustafsson et al., 2009). Concerning the inner capacitance of gibbsite, the value used by Gustafsson (2001) was selected. The outer capacitance of this Al-oxide was set to the value used for Fe-oxides based on the similarity of the inner capacitances between Fe- and Al-oxides, because of the lack of consistent value for the type of electrolyte solution used in the present study (Sahai and Sverjensky, 1997). With respect to kaolinite and illite, the inner and outer capacitances values proposed by Lackovic et al. (2003) were used. Note that the same capacitance values were used to fit the published experimental data that enabled the determination of the original surface complexation constants (see below) and to fit P adsorption in the studied soil.

Three types of P innersphere complexes, as observed by infrared spectroscopy (e.g. Tejedor-Tejedor and Anderson, 1990; Persson et al., 1995), were considered. The first type ($\equiv SOPO_3^{2.5-}$) is called monodentate surface complex as it shares only one ligand with the binding sites. In contrast, bidentate and protonated bidentate surface complexes ($\equiv SO_2PO_2^{2-}$ and $\equiv SO_2POOH^-$, respectively) share two ligands with two surface hydroxyl sites. These surface complexes have the same reaction stoichiometry and charge distribution for all studied minerals (Gustafsson, 2001; Manning and Goldberg, 1996), except the monodentate complex that is biprotonated once formed at the surface of gibbsite (Gustafsson, 2001).

Table 2

(a) Properties of soil minerals and soil organic matter and (b) model parameters used to simulate the concentration of inorganic phosphorus extracted with $CaCl_2$. Values in italics are from the present study. >RCO represents the reactive surface sites of natural organic matter, $\equiv$SO stands for a reactive surface hydroxyl bound to a metal (Fe or Al) at the surface of a metal oxide mineral or an aluminol group outcropping at the clay mineral edges, $\equiv X^-$ represents the permanent negatively charged sites on basal plane of clays.

	Goethite	Ferrihydrite	Gibbsite	Kaolinite	Illite	Carboxylic	Phenolic
(a)							
Concentration ($g\,l^{-1}$)	6.174	0.324	1	35.12	39.94	–	–
Specific surface area ($m^2\,g^{-1}$)	105[a]	750[c]	54[e]	23.6[g]	26.19[k]	–	–
Site density ($site\,nm^{-2}$)	6.15[a]	4[c]	4[c]	0.82[g]	2[k]	–	–
Site density ($mmol\,kg^{-1}$)	–	–	–	4.5[h]	6.25[j]	–	–
Point of zero charge ($\log K_1$)	9.2[a]	8.5[c]	10[c]	4.36[i]	3.46[k]	–	–
Innersphere capacitance ($F\,m^{-2}$)	0.9[b]	0.9[d]	1.1[c]	1.5[j]	1.5[j]	–	–
Outersphere capacitance ($F\,m^{-2}$)	0.74[b]	0.74[d]	0.74[f]	5[j]	5[j]	–	–
Q_{max} ($mol\,kg^{-1}$)	–	–	–	–	–	3.35[l]	2.55[l]
nH	–	–	–	–	–	0.81[l]	0.78[l]
nCa	–	–	–	–	–	0.63[l]	0.75[l]
p	–	–	–	–	–	0.49[l]	
(b)							
Thermodynamic equilibrium constant (log K_2)							
Surface complexes							
$\equiv SOPO_3^{-2.5}$	20.8[a]	19.7[c]	–	*20.8*	*17.44*	–	–
$\equiv SO_2PO_2^{-2}$	25.59[a]	26.01[c]	25.45[c]	*25.5*	*26*	–	–
$\equiv SO_2POOH^-$	31.79[a]	32.61[c]	33.05[c]	*32.6*	*30*	–	–
$\equiv SOPO_3H2^{-0.5}$	–	–	32.5[c]	–	–	–	–
$\equiv SOCaOH^{-0.5}$	−8.63[m]	−8.63[d]	*−15*	–	*−8*	–	–
$\equiv SO_2CaOH$	–	–	*−15*	*−7*	–	–	–
$\equiv SO_2Ca$	–	–	*4*	–	*1.5*	–	–
$\equiv SOCa^{1.5}$	–	–	–	*3.1*	*3.1*	–	–
$2{\equiv}X^- \cdot Ca$	–	–	–	*0.32*	*0.62*	–	–
$\equiv SOK^{0.5}$	–	–	–	−2.5[g]	–	–	–
$\equiv SONa^{0.5}$	–	–	−2.7[n]	–	−2.7[o]	–	–
$\equiv SOCl^{-0.5}$	7.1[a]	7.1[c]	7.8[n]	7.9[g]	7.9[o]	–	–
>RCOH	–	–	–	–	–	2.93[p]	8[p]
>RCOCa	–	–	–	–	–	−1.37[l]	−0.43[l]

[a] Hiemstra and Van Riemsdijk (1996).
[b] Hiemstra and Van Riemsdijk (2006).
[c] Gustafsson (2001).
[d] Assumed to be equal to those of goethite (see text).
[e] Manning and Goldberg (1996).
[f] Assumed to be equal to those of Fe-oxides (see text).
[g] He et al. (1997).
[h] Gu and Evans (2008).
[i] Duc et al. (2005).
[j] Lackovic et al. (2003).
[k] Liu et al. (1999).
[l] Milne et al. (2003).
[m] Rahnemaie et al. (2005b).
[n] Sahai and Sverjensky (1997).
[o] Gu and Evans (2007).
[p] Milne et al. (2001).

Therefore, only the equilibrium constants ($\log K_2$) vary with the nature of the mineral (see Table 2b).

Chloride is known to be adsorbed as an outersphere complex by mineral surfaces (Hiemstra and Van Riemsdijk, 1996; He et al., 1997; Gustafsson, 2001; Gu and Evans, 2007). The Ca can be either adsorbed as innersphere or outersphere complexes depending on the mineral. Calcium adsorption onto goethite and ferrihydrite has been comprehensively studied (e.g. Rahnemaie et al., 2005b). These works revealed that Ca adsorption onto such Fe-oxides occurred as innersphere monodentate surface complexes.

It was assumed that all the soil organic C corresponds to 'active' humic substances regarding metal binding and that these are entirely made of humic acids (e.g. Dudal and Gérard, 2004; Lumdson, 2004). The predominance of humic acids was also supported by the low concentration of dissolved organic C that was measured at the native soil pH (data not shown), which indicated the relative stability of the soil organic matter (Lofts et al., 2001). The parameters proposed by Milne et al. (2001, 2003) were used for Ca binding and acid–base properties of a generic humic acid.

2.4. Undetermined binding properties

In contrast with Fe-oxides, which are well-documented with respect to P and Ca binding properties (see Table 2b), the parameters for the adsorption of P and Ca onto clay minerals and the adsorption of Ca onto gibbsite are apparently lacking in the literature. To obtain these parameters, published experimental data measured in single mineral systems have been reinterpreted with the 1-pK TPM. The number of unknowns was also limited by considering that Ca and P adsorption involve the same reaction stoichiometry and charge distribution as other cations and anions that are known to exhibit similar adsorption properties (Manning and Goldberg, 1996; Ponthieu et al., 2006; Sverjensky, 2006). Therefore, only the equilibrium constants of the different surface reactions were estimated here.

Concerning the adsorption of P onto kaolinite and illite, the charge distribution and reaction stoichiometry determined for As(V) adsorption by Manning and Goldberg (1996) was used. These authors considered three types of innersphere complexes: monodentate, bidentate, and protonated bidentate. Equilibrium constants associated with P adsorption reactions at kaolinite and illite surfaces were fitted from experimental data reported by Dimirkou et al. (1997) and Manning and Goldberg (1996), respectively. In these previous works, KCl or NaCl were used as background electrolytes. Equilibrium constants for the adsorption of K and Na onto these clays are given in Table 2b. These monovalent cations form outer-sphere complexes (Rahnemaie et al., 2005a).

The adsorption of Ca onto Al-oxides mineral has already been described using innersphere bidentate surface complexes (Sverjensky, 2006), which would differ by the number of protons released from the mineral surface during their formation (ranging from 0 to 2). Their equilibrium constants were obtained by fitting experimental data provided by Huang and Stumm (1973).

Concerning the complexes formed during Ca adsorption onto kaolinite and illite, spectrometry data on the adsorption of Cd by Lackovic et al. (2003) were reinterepeted. The consideration of data relative to Cd adsorption as a proxy for Ca is in agreement with the crystal chemical theory which stipulates that different divalent cations form similar innersphere and outersphere complexes on edge sites of clays. Calcium adsorption was simulated on permanent negative charged sites by ion-exchange with the release of two protons, as commonly made with various divalent cations (Lackovic et al., 2003; Gu and Evans, 2008). Accordingly, the adsorption of Ca on the edge sites of kaolinite was controlled by the formation of an outersphere bidentate surface complex and an innersphere bidentate surface complex associated with the release of two protons. The exchange reaction between Ca and two protons was also taken in account on permanent negative charged sites of this clay mineral. Thermodynamic equilibrium constants for these reactions were obtained by modelling the zeta potential of kaolinite measured after the adsorption of different quantities of Ca (Vane and Zang, 1997). The electrostatic potential of the head end of the diffuse layer (2-plane) was linked to the zeta potential using the following equation (Zarzycki et al., 2007):

$$\zeta = \psi_d \exp(-\kappa Z) \tag{10}$$

where ζ is the zeta potential (V), κ is a constant (termed as the Debye's parameter) and Z is the distance between the electrostatic plane of the 2-plane and the plane of shear, which is related to the ionic strength.

Concerning the adsorption of Ca onto illite, the formation of a single innersphere monodentate surface complex and two bidentate surface complexes, an outersphere and an innersphere surface complexe, was considered. As for kaolinite, the exchange between two protons and Ca was considered on the permanent negatively charged sites (Gu and Evans, 2007). The experimental data supplied by Konan et al. (2007) was simulated in an attempt to determine the equilibrium constants for these surface reactions.

2.5. Statistics

The statistical analyses were performed with the R software (CRAN, 2006). The goodness-of-fit of the simulation of the concentrations in P–$CaCl_2$ vs. soil pH was estimated by Pearson's correlation coefficient and the Root Mean Square Error (RMSE):

$$\text{RMSE} = \sqrt{\sum_{i=n}^{1} \frac{(X_{o,i} - X_{c,i})}{n}} \tag{11}$$

where n is the number of samples, corresponding to different soil pH values, $X_{o,i}$ is the measured P concentrations and $X_{c,i}$ is the concentration calculated by the model.

3. Results

3.1. Estimation of missing binding properties

Results of the simulations of P adsorption data for kaolinite and illite are shown in Fig. 2a and b. The model adequately reproduced the measured concentrations of P adsorbed onto kaolinite at different pH values ($R^2 = 0.85$, RMSE = 0.1 µmol g^{-1}). Experimental data relative to the adsorption of P onto illite were also simulated quite accurately ($R^2 = 0.87$, RMSE = 0.035 mmol kg^{-1}). Note that the adsorption of P onto these two clay minerals decreased from pH 6 toward alkaline pH values (by 40% and 80% for kaolinite and illite, respectively). Under more acidic conditions, kaolinite adsorbed a quasi-constant concentration of P, i.e. a plateau was reached from pH 6 toward more acidic pH values. In contrast, the concentration of P adsorbed onto illite decreased markedly with decreasing pH below pH 6.

Experimental and simulated results relative to Ca adsorption onto gibbsite, kaolinite and illite are presented in Fig. 3. By adjusting the equilibrium constants for the adsorption of Ca onto gibbsite, the model reproduced reasonably well the experimental data ($R^2 = 0.8$, RMSE = 0.3 µmol m^{-2}). From neutral to mildly alkaline conditions (pH 9.5), quite good agreement was obtained between measurements and modelling results. Above pH 9.5, the model progressively underestimated proton concentrations that are competitively released by Ca adsorption. The discrepancy reached 16% for hyperalkaline conditions (pH 11). The model also reproduced quite accurately ($R^2 = 0.9$, RMSE = 0.6 mV) the zeta potential of kaolinite as measured by Vane and Zang (1997) over Ca concentrations ranging from 1 to 1000 mg l^{-1} (Fig. 3b). Regarding the simulation of experimental conditions for Ca adsorption onto illite (Konan et al., 1997), the model was also able to satisfactorily reproduce the concentration of adsorbed Ca until saturation

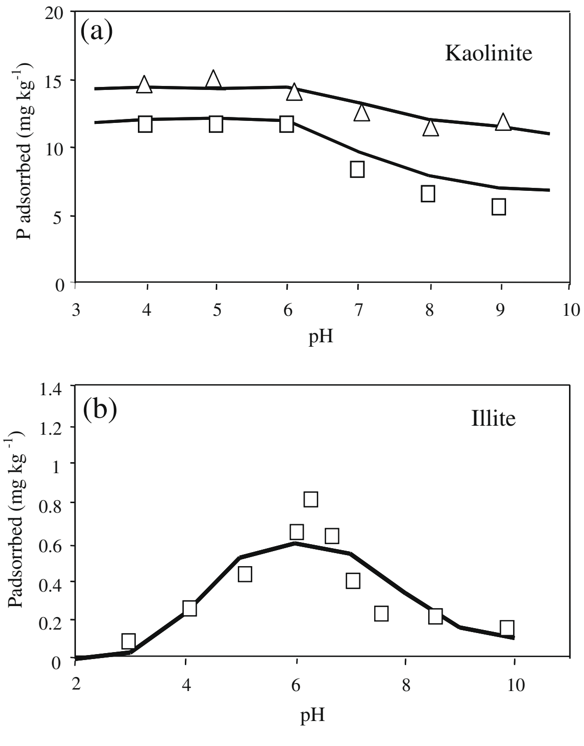

Fig. 2. Relationship between pH and concentration of P adsorbed onto (a) kaolinite in 0.1 M KCl at two total P concentrations (40 and 89 µM), measured by Dimirkou et al. (1997) and (b) illite in 0.1 M NaCl and at a P concentration of 6.7 µM, from Manning and Goldberg (1997). Symbols represent experimental data and the continuous line corresponds to the modelling results.

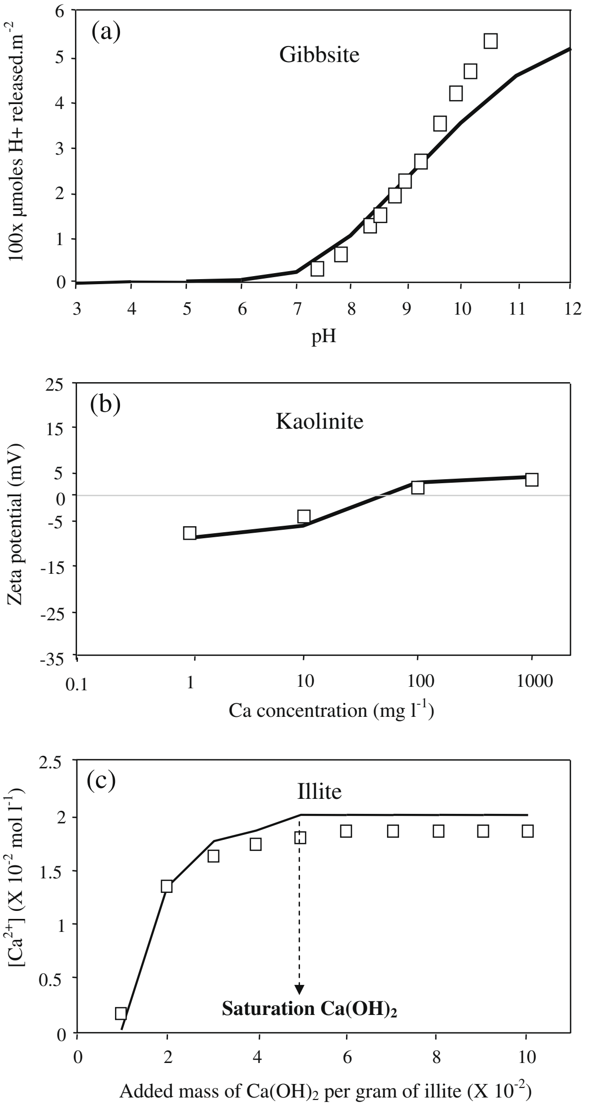

Fig. 3. (a) Proton release by gibbsite caused by Ca adsorption as a function of pH in 0.1 M NaCl at a Ca concentration of 1 mM (Sverjensky, 2006); (b) zeta potential of kaolinite in 0.01 M KCl as a function of dissolved Ca concentration (Vane and Zang, 1997); (c) Ca concentrations in solution in presence of illite at increasing total Ca concentrations (Konan et al., 2007). Symbols represent experimental data and the continuous line corresponds to the modelling results.

(Fig. 3c) with respect to Ca hydroxide ($R^2 = 0.9$, RMSE = 0.05 mmol l^{-1}). Note that the thermodynamic equilibrium constant defined by Konan et al. (2007) was used for Ca hydroxide ($\log K = 32.70$).

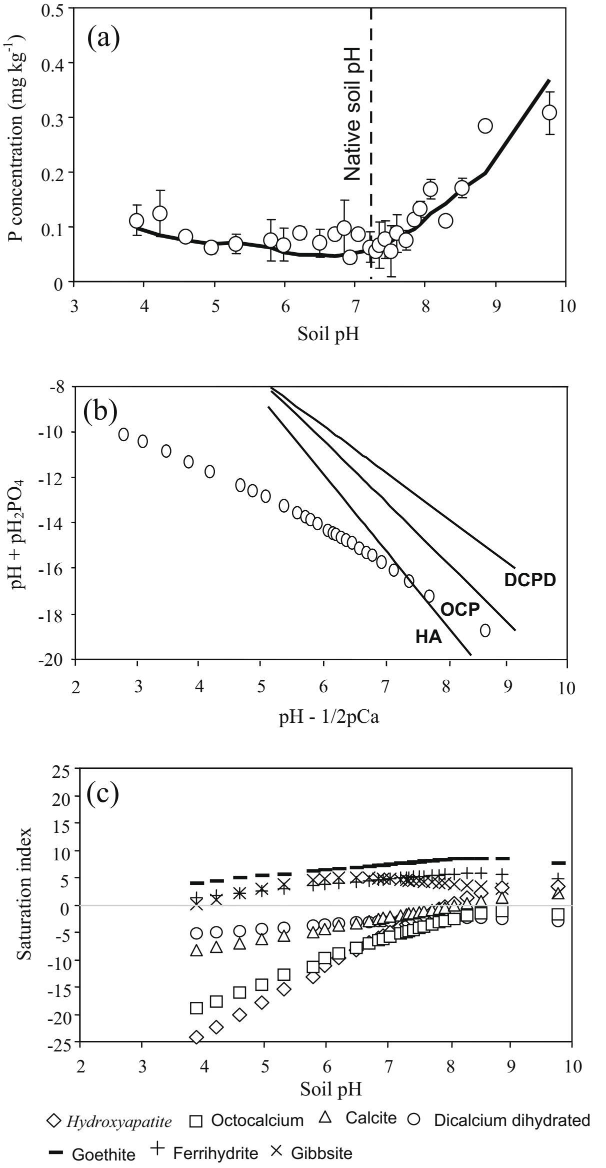

Fig. 4. (a) Relationship between soil pH and concentration of P extracted with $CaCl_2$ (0.01 M) of the surface horizon of the studied soil (Chromic Cambisol, Cazevieille, France). The soil was acidified by adding H_2SO_4 and alkalinised by adding KOH. The symbols stand for the measured data, with the error bars corresponding to ±standard deviation ($n = 3$). The continuous line corresponds to simulated values ($R^2 = 0.80$, RMSE = 0.03 mg kg^{-1}). (b) Stability diagram for three potentially precipitating Ca-phosphate minerals: dicalcium dehydrated (DCPD), octacalcium (OCP) and hydroxyapatite (HA), showing the equilibrium state of the solutions extracted with $CaCl_2$ (symbols). (c) Saturation Index of Al- and Fe-oxides, calcite and P-minerals as a function of soil pH. Aqueous Fe and Al concentrations were determined from their concentration measured in the CEC. Iron is assumed as Fe^{3+}. Partial pressure of CO_2 was set to the value in the atmosphere ($10^{-3.5}$ atm).

3.2. Geochemical processes

Fig. 4a shows the measured values of P–$CaCl_2$ as a function of soil pH. The different amounts of added alkali or acid have resulted in pH values ranging from 3.9 to 9.8, while the initial, untreated soil pH was 7.2. A little increase in P–$CaCl_2$ was observed at $pH < 5.8$ and a more pronounced increase above pH 7.4. Compared with the value at native pH, P–$CaCl_2$ concentrations increased up to a factor of 7 under alkaline conditions (pH 9.8). It can also be seen in Fig. 4a that the model accurately simulated P–$CaCl_2$ over the entire range of soil pH ($R^2 = 0.8$, RMSE = 0.03 mg kg^{-1}). In the acidic and neutral pH range ($pH < 7.4$), simulated data were very close to the actual concentrations ($R^2 = 0.85$, RMSE = 0.02 mg kg^{-1}). At the two most alkaline soil pH values (pH 8.9 and 9.8), the discrepancy between simulated and measured concentrations was slightly larger ($R^2 = 0.7$, RMSE = 0.08 mg kg^{-1}).

The stability diagram made for Ca-phosphate minerals (Fig. 4b) showed us that the solutions extracted with $CaCl_2$ were undersaturated with respect to the less soluble Ca-phosphate form (hydroxyapatite) at $pH < 8$, approximately. At larger pH, the extracted solutions were oversaturated with respect to hydroxyapatite but remained undersaturated with respect to octacalcium phosphate and dicalcium phosphate dihydrate.

Similarly to hydroxyapatite, the solutions extracted with $CaCl_2$ below pH 8 were undersaturated with respect to calcite (Fig. 4c).

It was also calculated that Al- and Fe-oxides were oversaturated over the investigated pH range. Note that kaolinite and illite saturation indexes were not calculated since Si concentration was not measured in the experiments.

3.3. Phosphorus and electrolyte adsorption mechanisms

Geochemical modelling enabled the study of the contributions of different soil constituents: (i) to P adsorption and to (ii) Ca and Cl adsorption (i.e. adsorption of electrolyte ions) in the studied soil.

Iron-oxides were the major P-adsorbing phases at acid and alkaline pH values. The contribution of Fe-oxides increased quite sharply at alkaline pH (at pH > 8). Under these conditions, Fe-oxides adsorbed up to 70.4 mg of P per kg of soil; i.e. 53% of the total adsorbed P (Fig. 5a). As the soil pH increased from acid to neutral values, P adsorption was progressively controlled to larger extents by clays and particularly illite than by Fe- and Al-oxides.

Fig. 5b shows that the model simulated an increase of Ca adsorption by the soil organic matter and that adsorption by minerals also increased as the soil pH increased. From the most acid to the most alkaline conditions, the concentration of adsorbed Ca increased from 3% to 13% of the total Ca concentration. Calcium was essentially adsorbed by soil organic matter, which is assumed to be made of humic acids. Over the entire pH range, the percentage of Ca adsorbed by the humic acid remained quite stable, ranging from 86% to 91% of the total adsorbed Ca. Illite adsorbed the largest Ca concentration, which remained almost steady over the entire pH range (Fig. 5d). In contrast, the adsorption of Ca onto kaolinite increased substantially with increasing pH. Iron and Al-oxides adsorbed significant amounts of Ca at alkaline pH only. Amongst soil minerals, goethite contributed much more to Ca adsorption, as this Fe-oxide adsorbed a maximum of 190 mg kg^{-1} of Ca whereas gibbsite and ferrihydrite adsorbed only 11 and 48 mg kg^{-1}, respectively.

The concentrations of adsorbed Cl calculated by the model are plotted in Fig. 5c. The concentration decreased from 142 mg kg^{-1} to 25 mg kg^{-1} as the pH of the soil samples increased from acidic to alkaline conditions. On average, adsorbed Cl amounted to about 3% of the Cl concentration in the electrolyte solution. Illite exhibited the largest concentrations of adsorbed Cl and the adsorption of Cl onto kaolinite was important at acidic pH only. In contrast, Al- and Fe-oxides always adsorbed traces of Cl.

The distribution of the three P surface complexes (monodentate, bidentate and protonated bidentate) can also be modelled (Fig. 6). Over the entire explored pH range, P was predominantly adsorbed as monodentate surface complexes (about 73 mg kg^{-1}, i.e. 54% of the total adsorbed P). Protonated bidentate surface complexes occurred only at acidic pH but contributed weakly to the surface speciation. The maximum concentration of P adsorbed as protonated bidentate surface complexes amounted to 12.6 mg kg^{-1} (about 10%) at the most acidic soil pH (pH 3.9). The concentrations of bidentate and monodentate surface complexes were equivalent from pH 6.5 to 8. At pH > 8, monodentate complexes predominated, representing up to 80% of the total adsorbed P, reaching 113 mg kg^{-1} at the most alkaline soil pH (pH 9.8).

3.4. Relationship between the adsorption of cations and anions

Fig. 7a illustrates the variations in the concentrations of Ca and P adsorbed onto illite as a function of its surface charge. It can be seen that both P and Ca adsorption increased with increasing illite surface charge although these ions have opposite charges. Furthermore, adsorbed Ca increased by 25% while adsorbed P increased by 68% when illite surface charge varied from 0.31 to 0.67.10^{-3}

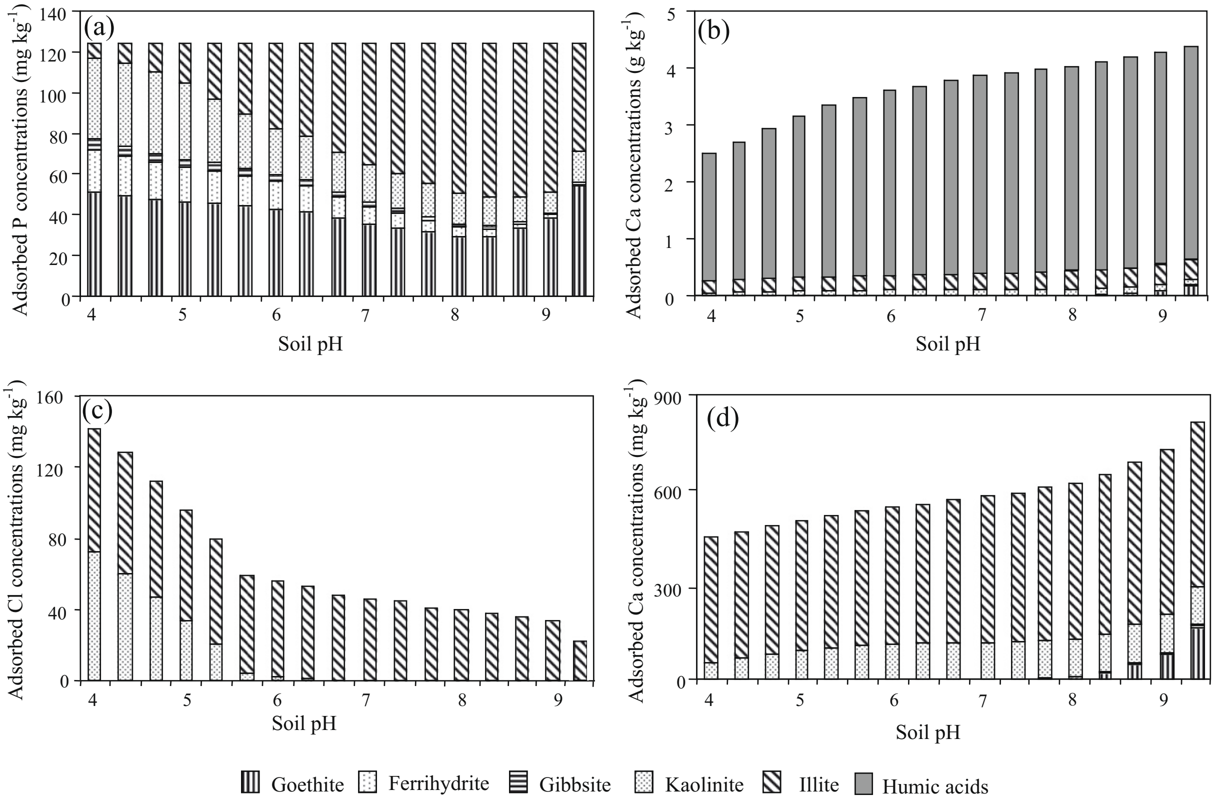

Fig. 5. Concentrations of P (a), Ca (b) and Cl$^-$ (c) adsorbed onto the different constituents of the studied soil (Chromic Cambisol, Cazevieille, France) as a function of soil pH. (d) Focus on the concentration of calcium adsorbed onto the different soil minerals.

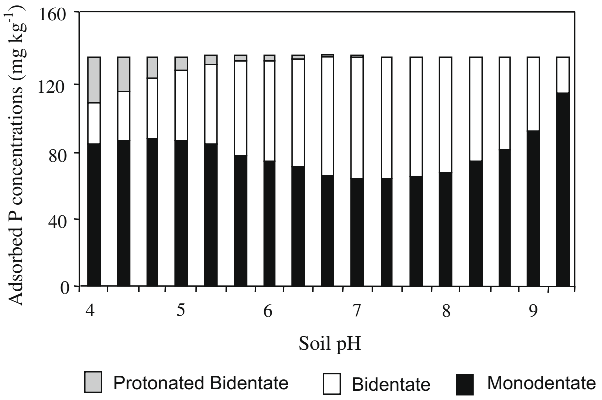

Fig. 6. Distribution of P surface complexes as a function of soil pH.

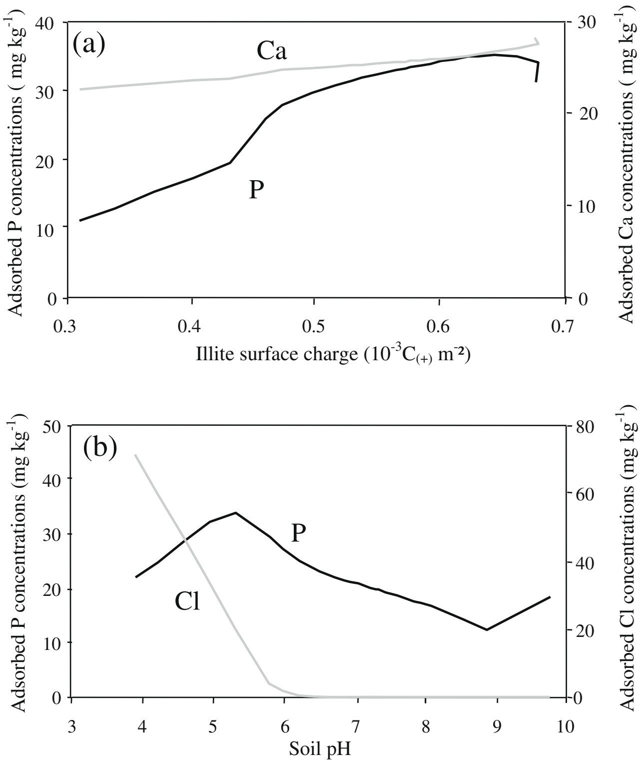

Fig. 7. (a) Concentrations of P and Ca adsorbed onto illite as a function of its surface charge; (b) concentration of P and Cl^- adsorbed onto kaolinite as a function of soil pH.

$C_{(+)}m^{-2}$. This indicates that the adsorption of Ca was less sensitive to a variation of illite surface charge than the adsorption of P.

Fig. 7b shows the concentrations of P and Cl adsorbed by kaolinite as a function of soil pH. Under acidic conditions, the concentrations of adsorbed P and Cl exhibited opposite behaviours. The concentration of P adsorbed onto kaolinite increased up to pH 5.4, with a maximum value of 34 mg kg^{-1}, while the concentration of adsorbed Cl decreased from 72 mg kg^{-1} to zero.

4. Discussion

4.1. Controlling geochemical process

The simulation of P–$CaCl_2$ performed with mechanistic adsorption models provided adequate results. This success suggests that the adsorption process controlled the EAP of the studied soil over a large range of pH conditions around the native pH. This finding is further confirmed by the fact that the degree of P saturation (i.e. the ratio between P concentration and the amount of Al and Fe extracted by ammonium oxalate) was low, as defined by Litaor et al. (2003) and McDowell et al. (2003) for soils where adsorption seems to be the predominant geochemical process controlling EAP. The undersaturation of phosphate minerals obtained from pH 4 to about pH 8 also reinforces the finding that adsorption processes controlled EAP in the studied soil (see Fig. 4b). Under alkaline conditions, the formation of the least soluble Ca-phosphate tested here (hydroxyapatite) may explain the modest difference between measured and calculated P–$CaCl_2$ concentrations (see Fig. 4a). Such a shift with soil pH in the geochemical process controlling the EAP has already been reported by McDowell et al. (2003). It was also found that P–$CaCl_2$ extracts were always oversaturated with respect to Fe- and Al-oxides thus precluding any effect of their dissolution (Fig. 4c). The influence of the precipitation of Fe- and Al-oxides is negligible, as is commonly assumed in adsorption studies performed under similar pH and temperature conditions (e.g. Hiemstra and Van Riemsdijk, 1996, 2006; Gustafsson, 2001). By the same token, the influence of clay dissolution or precipitation on the adsorption process can be neglected (e.g. Gu and Evans, 2007, 2008).

4.2. Validity of estimated binding properties

The results of the modelling also suggests that the three surface complexes involved in the adsorption of As(V) onto kaolinite and illite (Manning and Goldberg, 1996) can also be used to describe P adsorption. Furthermore, the fitted equilibrium constant values for P adsorption (see Table 2b) were larger than those reported by Manning and Goldberg (1996) for As(V) for a constant-capacitance model, which are the only data available for comparison purposes. Such comparison between metal binding constants that are consistent with the 1-pK TPM and those consistent with the constant-capacitance model has already been made by Lackovic et al. (2003) and Gu and Evans (2007, 2008). Consistently, such higher affinity of P relative to As(V) for adsorption by clay surfaces was also obtained by Violante and Pigna (2002). This may be due to the lesser negative charge brought in by P when it is adsorbed, thereby favouring its adsorption.

Strong agreement was also obtained by modelling published results of Ca adsorption onto gibbsite, kaolinite, and illite. This result suggests that the innersphere and outersphere surface complexes reported for other divalent metal cations can be used to simulate Ca adsorption (Lackovic et al., 2003; Rahnemaie et al., 2005b; Sverjensky, 2006). The equilibrium constants for Ca adsorption onto gibbsite were higher than those obtained by Sverjensky (2006) for Mg adsorption with the same electrostatic adsorption model. In the same way, the equilibrium constants for Cd adsorption onto kaolinite and illite proposed by Lackovic et al. (2003) were higher than those calibrated here for Ca adsorption. These differences in the log K_2 for Ca complexes relative to those for Cd and Mg complexes are in agreement with the crystal chemical theory stipulating that the equilibrium constant increases with increasing ionic radius of the adsorbed cation (Zhang et al., 2004; Rahnemaie et al., 2005b; Sverjensky, 2006).

Lastly, it was observed that the equilibrium constants for Ca adsorption on permanent negatively charged sites of illite and kaolinite were close to the values found for the exchange between Ca and Na by Tournassat et al. (2007) and Kopittke et al. (2006).

4.3. Distribution of adsorbed P: mineral composition and electrolyte influences

Results showed that the contribution of the various soil minerals to P adsorption changed with soil pH (see Fig. 5a). In particular, significant contribution of clay minerals to the adsorption of P between pH 5.8 and pH 7.4 was found. Such a high affinity of clay for P adsorption had also been observed by Manning and Goldberg (1996). The present work provides quantitative insights in the relative contribution of clay minerals and Fe- or Al-oxides and showed an unexpected, substantial contribution of kaolinite and illite over the large, investigated pH range, with illite being an important component. Modelling also revealed the importance of the background electrolytes on the adsorption properties of these clay minerals. First, the concentration of P adsorbed onto illite and to a lesser extent onto kaolinite increased with increasing soil pH, although deprotonation of the surface hydroxyl sites increased, thus leading to more anion-repulsive surfaces. This can be explained by surface deprotonation being counterbalanced by Ca adsorption, which resulted in positive charges in the double-diffuse layer. Such a build up of surface positive charges led to enhance P adsorption (see Fig. 7a). Therefore, the P–$CaCl_2$ concentrations remained stable in the range of neutral pH. At alkaline soil pH, Ca adsorption did not compensate for the deprotonation of mineral surfaces and thus P–$CaCl_2$ concentrations increased. This promoting mechanism for P adsorption has already been observed with Al/Fe-oxides and clay minerals (Rietra et al., 2001; Masue et al., 2007). Consistently, numerical simulations indicated that the adsorption of Ca onto illite and kaolinite is particularly important (see Fig. 5d). The high intensity of this mechanism for illite has also been observed by Smith et al. (2002), who studied As(V) adsorption in the presence of Ca. Such a great affinity of Ca toward illite and kaolinite binding sites should be ascribed to their low PZC, relative to Fe- and Al-oxides (see Table 2b).

It was also calculated by modelling that the adsorption of P decreased at very acidic soil pH, leading to larger values of P–$CaCl_2$ (see Fig. 4a). This result was rather surprising because of the increasing protonation of mineral surfaces with decreasing pH conditions, which would imply an increased adsorption of P (Hiemstra and Van Riemsdijk, 1996; He et al., 1997). Decreasing P adsorption under acidic conditions had however also been observed by Geelhoed et al. (1997) and Hiemstra and Van Riemsdijk (1998). This effect appears even more pronounced with clay minerals (He et al., 1997). Again, the influence of the background electrolyte can explain this result. The numerical simulation revealed that kaolinite adsorbed significant amounts of Cl only at acidic pH values, i.e. below pH 6 (see Fig. 5c). Moreover, the adsorption of this monovalent anion increased with decreasing pH and the increase in Cl adsorption was quite well correlated (in the acidic pH range) with the decrease in P adsorption onto this clay mineral (see Fig. 7b). Consequently, such an inverse correlation between adsorbed Cl and adsorbed P may be indicative of a competition between these two anions toward kaolinite binding sites, ultimately resulting in increased P–$CaCl_2$ at acidic pH.

The concentration of P adsorbed onto gibbsite and Fe-oxides varied with soil pH with a similar pattern to P–$CaCl_2$. Indeed, the concentration of P adsorbed onto Al- and Fe-oxides increased in both the acidic and alkaline pH domains, where these minerals contributed to as much as 50% of the total adsorbed P, the remaining part being bound to clay minerals. At acidic pH, the large contribution of Fe-oxides and gibbsite can be explained by their high PZC, especially for gibbsite (see Table 2a). At alkaline pH, those minerals replaced clay minerals in playing a major role in P adsorption due to their high surface charge, despite that Ca adsorption onto clay minerals occured (see above). In spite of this, a marked increase in P–$CaCl_2$ was observed (see Fig. 4a). Such P desorption at alkaline pH has been widely described in different single mineral systems (e.g. Barrow et al., 1980; Hiemstra and Van Riemsdijk, 1998). This was caused by the large extent of the deprotonation of all mineral surfaces under these pH conditions, which was apparently not compensated for by the additional positive charges accompanying the adsorption of Ca onto Al- and Fe-oxides.

4.4. Influence of phosphorus surface complexes

The slight increase in P–$CaCl_2$ under acidic pH may also be related to the strength of P surface complexes. The numerical simulation indicated that protonated bidentate complexes contributed significantly to P adsorption in the acidic pH range only (see Fig. 6). Because of the Boltzman factors (e.g. Rietra et al., 2000), the formation of this type of surface complex adds more negative charges onto mineral surfaces than bidentate and monodentate complexes, which dominated surface complexation under less acidic and near neutral conditions. Such additional negative charges increased the repulsive interactions between P and mineral surfaces, which led to decreasing P adsorption.

Monodentate surface complexes dominated P surface speciation under alkaline pH conditions (see Fig. 6). Their formation resulted in less negative charges at the mineral surfaces than bidentate surface complexes and thus decreased the repulsion of anions. However, it appeared that this mechanism, inasmuch as that associated to the adsorption of Ca, was not efficient enough to counterbalance the effect of the deprotonation of binding sites, which led to increased P–$CaCl_2$ under these pH conditions (see previous section).

The numerical modelling also revealed that monodentate complexes are the most abundant P surface complexes for goethite over the entire pH range. This was not observed in a study performed by Rietra et al. (2001) in solutions containing Ca and P. The predominance of monodentate species may be due to the low saturation of goethite surface sites (7.5%) achieved in the present work. The low saturation of goethite surface sites led to decreasing repulsive effects, which promoted the formation of monodentate complexes that are more stable than bidentate complexes under these conditions (Rose et al., 1997). Nevertheless, spectrometric data such as provided by EXAFS are required to identify the actual nature of the surface complexes (Tejedor-Tejedor and Anderson, 1990).

5. Conclusions

Mechanistic adsorption models (1-pK Triple Plane Model, ion-exchange and NICA-Donnan) were used to simulate the concentration of environmentally available P (EAP) in the topsoil of a Chromic Cambisol over a range of pH values. Strong agreement was found with the experimental data. In this modelling investigation, the properties of the individual minerals and those of the soil organic matter were considered through an additive approach. The concentration of inorganic P extracted by $CaCl_2$ (P–$CaCl_2$) was used as an indicator of the EAP. Based on thermodynamic equilibrium considerations, it appears that the dissolution–precipitation of Ca-phosphate minerals did not control the EAP over the investigated range of pH values (from pH 4 to 10). This process may have played a role only under the most alkaline pH conditions, explaining the moderate overestimation of P–$CaCl_2$ by the model at such pH. This work made it possible to rank the contribution of different soil minerals to P adsorption and to show an unexpected, major contribution of clay minerals, except in the most acidic conditions. The use of $CaCl_2$ as background electrolyte required to account for the adsorption of Ca and Cl by soil constituents, which were found to exert a promoting and competing effect on P adsorption, respectively. The influence of the distribution of P surface complexes on the variation of P–$CaCl_2$ as a function of pH was highlighted as well. A major finding provided by the model is that while P adsorption and EAP varied little over a large range of pH values (5–7.5, or even 4.5 to about 8 according to measured values), the relative contribution of the various minerals and binding sites did vary considerably. From a functional point of view, this means that in this pH range, any pH change would thus result in P ions desorbing from sites or mineral surfaces and re-adsorbing onto some other. This may provide a chance for plant roots that are capable of altering the rhizosphere pH to recover some of these P ions in spite of the apparent little change of EAP with pH.

This study thus demonstrated: (i) the possibility of modelling complex systems in general, and in the present case of geochemical modelling, with generic parameter values as a unique tool to understand complex, interacting physico-chemical processes in soils; (ii) the applicability of mechanistic adsorption models to a 'real' soil, with its mineralogical complexity and the additional contribution of soil organic matter. All model parameters were taken from the literature or obtained by fitting published experimental values in single mineral systems. Indeed, the proposed mechanistic modelling is a promising approach to model the EAP and study a number of environmental issues related to P dynamics in soils.

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Which Formalism to Model the pH and Temperature Dependence of the Microbiological Processes in Soils? Emphasis on Nitrification

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Abstract We compared three formalisms derived from (1) medical sciences, (2) environmental, and (3) agronomic models to simulate the effects of pH and temperature on the nitrification rate in arable soils. We evaluated these formalisms by implementing them in a simple growth model by comparing modelled and measured nitrate contents of soil samples with different initial soil pH after incubation at two temperatures. We found that the formalisms derived from medical sciences were more accurate and easily adapted to the data, suggesting that such formalisms may be profitably used in agronomic or environmental models.

Keywords Microbial growth · Model · Formalisms · Environment · Soil

1 Introduction

Modelling microbial growth kinetics is essential in many fields for purposes such as waste management, medicine, food or environment sciences. In agronomic or environmental models, the Monod and exponential growth models are commonly used to convert through

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a yield term the bacteria growth into a reaction rate. They both have their own formalisms to cope with the effect of important environmental factors such as pH and temperature.

In the Monod model, the pH dependence of bacterial growth is often described by using a modified half saturation constant (K_S). In the exponential growth model, the growth rate is weighted by a coefficient (C) ranging from 0 to 1, with 0 corresponding to a minimal pH and 1 to optimal pH for microbial growth. In these two models, K_S and C are determined empirically. In the Monod and exponential models, the temperature dependence of the reaction rate is often calculated according to the Arrhenius law or its derivative, the van't Hoff relationship (hereafter referred to as the Q_{10} law). A wide range of coefficients for the Q_{10} law can be found for the same reaction. Thus, the choice of the Q_{10} coefficient can be an important source of prediction uncertainties. Some authors were also dismissive of the application of the Q_{10} law to model complex biological processes [5]. In food or medical sciences, since the late 1980s, modellers have made considerable efforts to predict modification of bacterial growth rate as a function of pH, temperature, and water activity [9, 18, 19]. They proposed different formalisms than those previously presented herein which are based on cardinal parameters that explicitly take into account the minimal, maximal, and optimal values of pH and temperature. Therefore, these formalisms seem more comprehensive to account for the effect of pH and temperature on bacterial growth.

From the above summary, it arises that many empirical formalisms are available from different disciplines to describe the same effects, but no comparison of their accuracy has been done so far. In agronomic or environmental research, the most frequently modelled microbial process is nitrification because of its importance in plant nutrition, water quality, and greenhouse gas emission. Considering the pH effect on nitrification, models using the Monod description of nitrifier growth are based on different K_S values with a number of assumptions, which make the approach somehow unreliable [16]. Other models based on the exponential description of nitrifier growth use a coefficient which is usually deduced from quadratic [2] or linear [4] relationships. For the effect of temperature, the value of Q_{10} was found to range from 2 to 5 depending on the soil type [13]. The pH and temperature formalisms developed in medical and food sciences are not yet used in models devoted to agronomic and environmental applications.

The aim of the present work is to assess the feasibility and advantages of substituting the standard pH and temperature formalisms by those proposed in the food and medical sciences. Our reference microbial kinetics is the nitrification rate. We describe nitrifier growth by means of the exponential approach, and we tested (1) a cardinal pH and temperature formalism proposed by [11] and (2) an empirical formalism proposed by [4]. To broaden the scope of our study, we also compared results of modelling performed with the pH and temperature formalism used in the Monod approach for nitrifier growth [16].

2 Methodology

2.1 Model Descriptions

The changes in soil nitrate content with time are calculated from the nitrifier growth kinetics and by introducing a specific yield constant Y (Eq. 1). Several assumptions are made with respect to microbial growth. First, neither the lag phase of growth nor the maintenance energy is taken into account. Second, all cells are assumed to be initially active. Third, nitrogen (N) availability is not a limiting factor, and pH and temperature are considered as the only factors susceptible to modify growth rate.

It follows that the changes in $N\text{–}NO_3^-$ content vs. time can be expressed as:

$$\frac{d\left[N-NO_3^-\right]}{d\,t} = Y\frac{\mathrm{d}m}{\mathrm{d}t} \tag{1}$$

with:

$$\frac{d\,m}{d\,t} = \mu_{\text{actual}}m \tag{2}$$

where $[N\text{–}NO_3^-]$ is the nitrate content (mg kg^{-1} soil), Y is the specific yield constant (moles of $N\text{–}NH_4^+$ per cell, mol cell^{-1}), μ_{actual} is the nitrifier growth rate (h^{-1}) calculated at a given pH and temperature, and m is the number of nitrifiers per kilogram of soil (cell kg^{-1}).

2.1.1 First Formalism: Cardinal pH and Temperature Formalism

In this formalism, hereafter referred to as the CTP model, μ_{actual} is expressed as a function of μ_{opt}, which is the maximum growth rate observed at the optimum temperature (T_{opt}) and pH (pH_{opt}). This is done by weighting the value of μ_{opt} with two functions, f_1 and f_2. The exact expression for these functions depends on the temperature and pH conditions.

If $T_{\min}<T<T_{\max}$ and $pH_{\min}<pH<pH_{\max}$, one have:

$$\mu_{\text{actual}} = \mu_{\text{opt}}f_1f_2 \tag{3}$$

if $T<T_{\min}$ or $T>T_{\max}$ or $pH<pH_{\min}$ or $pH>pH_{\max}$:

$$\mu_{\text{actual}} = 0 \tag{4}$$

and, if $T = T_{\mathrm{opt}}$ and $pH = pH_{\mathrm{opt}}$:

$$\mu_{\mathrm{actual}} = \mu_{\mathrm{opt}}. \tag{5}$$

with:

$$f_1 = \frac{(T - T_{\max})(T - T_{\min})^2}{\left(T_{\mathrm{opt}} - T_{\min}\right)\left[\left(T_{\mathrm{opt}} - T_{\min}\right)\left(T - T_{\mathrm{opt}}\right) - \left(T_{\mathrm{opt}} - T_{\max}\right)\left(T_{\mathrm{opt}} + T_{\min} - 2T\right)\right]} \tag{6}$$

$$f_2 = \frac{(pH - pH_{\min})(pH - pH_{\max})}{(pH - pH_{\min})(pH - pH_{\max}) - \left(pH - pH_{\mathrm{opt}}\right)^2} \tag{7}$$

where $T_{\max}$ stands for the maximal temperature above which no growth occurs, $T_{\min}$ is the minimum temperature below which no growth occurs. The values of $pH_{\max}$ and $pH_{\min}$ are the maximum and minimum pH for growth to occur.

2.1.2 Second Formalism: A Linear Relationship for pH and Q_{10}

Two relationships have been proposed by [4] to account for the influence of the pH (function f_3) and temperature (function f_4) on the growth rate:

$$\mu_{\mathrm{actual}} = \mu_{\mathrm{opt}} f_3 f_4 \tag{8}$$

with:

$$f_3 = S(pH - pH_{\min}) \tag{9}$$

$$f_4 = Q_{10}^{\left(\frac{T - T_{\mathrm{ref}}}{10}\right)} \tag{10}$$

The f_3 function ranges from 0 to 1, with $\mu_{\mathrm{actual}} = 0$ if $pH < pH_{\min}$, and S is an experiment-derived factor. Concerning f_4, T, and T_{ref} are the actual and reference temperature, respectively.

2.1.3 Third Formalism: A Monod Approach

In this last method, the influence of the pH on the growth rate is described through an inhibition enzyme-like expression [16]:

$$\mu_{\mathrm{actual}} = \mu_{\mathrm{opt}} \left(\frac{\left[N - NH_4^+\right]}{K_{\mathrm{S}}\ f_5 + \left[N - NH_4^+\right]} \right) f_6 \tag{11}$$

with:

$$f_5 = \left(1 + \frac{10^{-pH}}{K_{\mathrm{I}}}\right) \tag{12}$$

$$f_6 = Q_{10}^{\left(\frac{T - T_{\mathrm{ref}}}{10}\right)} \tag{13}$$

where K_{I} and K_{S} (mol H^+ l^{-1}) are half saturation constants that cope with the presence and absence of H^+ inhibition, respectively. For f_6, the Q_{10} relationship is also used, as in the second formalism (see Eq. 10).

We calculated μ_{actual} and inserted the result in the exponential growth model (see Eq. 2) to be compared with the growth rates calculated by means of the two other formalisms.

2.2 Model Parameters

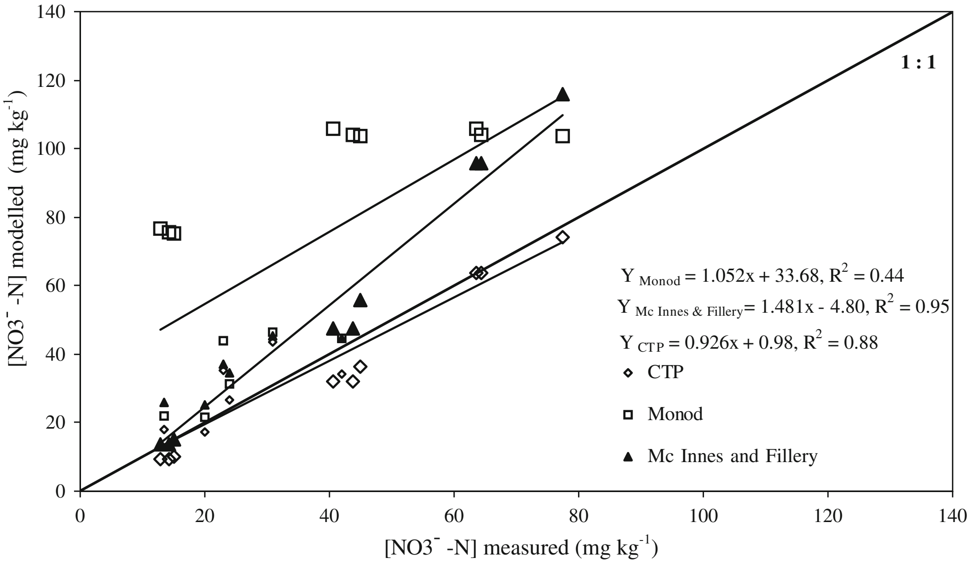
We calculated the nitrification rate and biomass over time (see Eqs. 1 and 2) by using values for Y and μ_{opt} specific to the *Nitrosomonas* species, which is one of the most common bacterium in arable soils [1]. A value of 1.05×10^{11} mol cell^{-1} was taken for Y [8]. We set the optimum growth rate, μ_{opt}, to 0.036 h^{-1} [1]. The initial value of the biomass density (m) was fixed at 2×10^8 cells kg^{-1} soil [16].

Different parameters are required depending on the tested formalism. In the CTP model, we set the optimum value for *Nitrosomonas* growth at pH 8 [8, 15]. In accordance with [14, 17], we used a minimum pH value of 4.5. Considering these values and a symmetric bell-shaped relationship between pH and growth [2], we calculated that the maximum pH for nitrification was 11.5.

In the second formalism [4], the minimum pH was also set at 4.5, and the value of S at 0.3. This latter formalism does not require maximum or optimum pH as input parameters. For the last formalism (Monod approach), authors [16] estimated pK_{I} to 6.3, with p$K_{\mathrm{I}} = -\log K_{\mathrm{I}}$. The K_{S} value for *Nitrosomonas* was 2.08 mg N–NH_4^+ l^{-1} (soil solution).

For temperature, we chose T_{opt} and T_{ref} of 30°C [7] and 20°C, respectively (see Eqs. 6 and 10). In the CTP model of [11], the maximum and minimum temperatures were derived from the generic relationships determined by [12]. We calculated $T_{\min} = -1$°C and $T_{\max} = 36$°C. In the two other formalisms, the Q_{10} value was set to 2.5 as an average of Q_{10} values found in the literature.

Fig. 1 Comparison of modelled and measured N–NO_3^- concentrations (mg kg^{-1}) for the acid soils (*oversized labels*) and alkaline soils (*minor-sized labels*) and for the three formalisms tested in the study. *Diamond labels*: CTP; *square labels*: Monod; *triangle labels*: McInnes and Fillery [4]

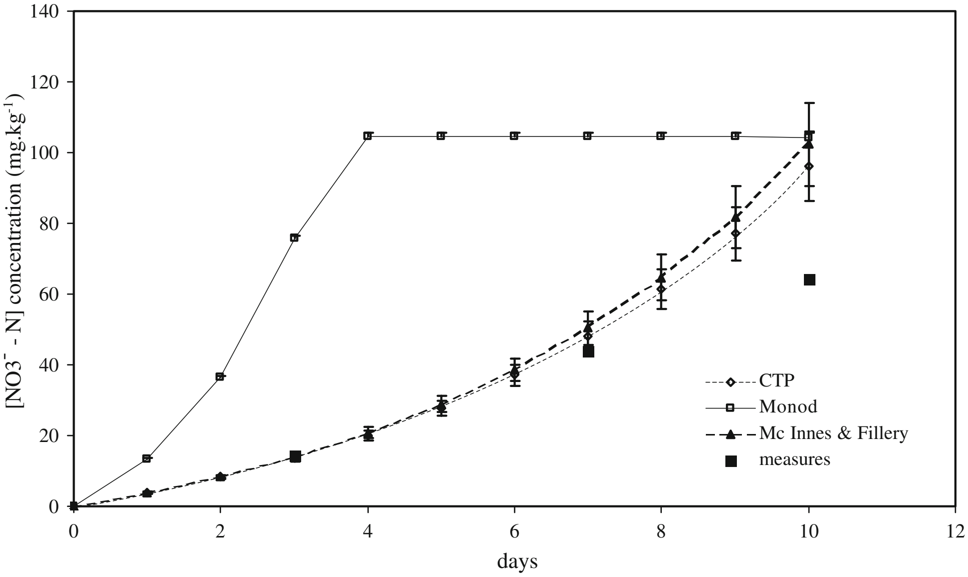


2.3 Simulation and Validation Dataset

For validation purposes, we selected two datasets from the literature [6, 10] and presented hereafter. Six different arable soils, three slightly alkaline (pH about 7.9) and three acid (pH about 5.6) soils, were sampled in northern and western France, from the 0–10 cm and 0–20 cm layers. Soil samples were 2-mm moist sieved and incubated at 10°C (alkaline soils) and 15°C (acid soils) after addition of ammonium, either as ammonium sulfate or diammonium phosphate and at two different concentrations (50 or 100 mg N kg^{-1}). Soil water content was carefully maintained during incubation at a value of pF 2.7. In these experiments, changes in the soil nitrate content over time were analyzed at 0, 2, and 6 days for alkaline soils and at 0, 3, and 7 days for acid soils.

Equations presented above were solved explicitly on a daily timestep over 7 days. Simulation ended when N–NH_4^+ concentration fell below 7 mg kg^{-1} soil. Feedback effects of pH variations subsequent to nitrification were not taken into account because, in the selected soils, no consequent pH variations were measured (around 0.5 pH unit). The simulated N–NO_3^- contents were compared to measured values using the software Statistica®, (Statsoft Inc, version 7.1, Tulsa, OK).

Fig. 2 Modelled nitrate concentration (mg kg^{-1}) over time calculated according to the three formalisms. Example given in acid soils. *Black squares*: measures; *diamonds*: CTP; *squares*: Monod; *triangles*: McInnes and Fillery [4]



3 Results and Discussion

Results showed that the CTP model was the most accurate formalism to describe the nitrification rate over the entire dataset (Fig. 1).

Indeed, the correlation between modelled and measured NO_3^- contents was good (R^2=0.88) and the linear regression parameters were close to the 1:1 isoline representing the best fit ($[N–NO_3^-]_{modelled}=0.98+0.926\ [N–NO_3^-]_{measured}$). The second formalism, proposed by [4], showed a smaller dispersion (R^2=0.95) but the regression parameters deviated considerably from the 1:1 isoline ($[N–NO_3^-]_{modelled}=-4.80+1.481\ [N–NO_3^-]_{measured}$). The linear regression curve obtained from the third formalism ($[N–NO_3^-]_{modelled}=33.68+1.052\ [N–NO_3^-]_{measured}$) was far from the 1:1 and the dispersion was high (R^2=0.44).

Taking into account the confidence limits of the linear regressions ($p<0.05$), we observed that the CTP formalism tended to slightly underestimate the nitrate content after 7 days in the three acid soils (oversized symbols) and in one alkaline soil at day 6 (minor-sized symbols). The CTP formalism also slightly overestimated the nitrate content for the two other alkaline soils at day 6 (Fig. 1, see minor-sized symbols). Using the second formalism, the simulated values fell within the confidence interval of the linear regression during the first simulation days (<4 days) whatever the soil pH. The discrepancy associated with the third formalism was substantial as the isoline is not contained within the confidence limits of the respective linear regression. In this latter formalism, the simulated nitrification ceased from day 4, explaining the alignment of data points along the *x*-axis (Fig. 2).

The third formalism tended to clearly underestimate the combined effect of the soil pH and temperature on bacteria growth for our dataset.

By calculating the values taken by the functions f_2, f_3, and f_5 involved in the different formalisms, we found that the latter function is less sensitive to pH variations. In none of the simulations and for all formalisms, the feedback effect of soil acidification by nitrifiers is taken into account, yet this should explain the errors in simulations after longer incubation times (6 to 10 days). The accuracy of the modelling differs within a given group of soils (i.e., acid or alkaline) as depicted in Fig. 1. Different explanations might account for this discrepancy, but the most probable is the existence of different amounts of nitrifiers (initial biomass, m_0) in the different soils, due to the history of the sampled field.

The three formalisms tested here require a knowledge of several parameters. In the CTP formalism, six parameters are involved (pH_{min}, pH_{opt}, pH_{max}, T_{min}, T_{max}, and T_{opt}). To reduce the number of parameters, we used the relationship proposed by [12]. For this, two parameters (T_{min} and T_{max}) are obtained from T_{opt}. Finally, the CTP formalism needs only four parameters to be estimated. The two other formalisms, i.e., McInnes and Fillery and Monod-like, required three parameters which were not so different from the CTP formalism. Moreover, the four remaining parameters in the CTP formalism are as simple to acquire as those involved in the other two formalisms. Note that the function f_1 increases slowly from T_{min} up to 15°C and more rapidly until T_{opt} and finally sharply decreases to T_{max}. Authors observed the same pattern of nitrification rates in acid soils [3]. The Q_{10} law cannot account for this pattern. However, all of the aforementioned formalisms are quite empirical, because they are based more or less by mathematical data fit of observed growth, especially when it comes to dealing with pH.

Since CTP better describes the physiological responses of bacteria to pH and temperature in our conditions and uses easily estimated parameters, we believe that the CTP formalism can be used to correct the microbial growth in soils for pH and temperature variations. Therefore, advances in food or medical microbiology should be a source of improvement for microbiologically mediated processes simulated in agronomic and environmental models.

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