

Sujet de thèse
École doctorale EEA de Lyon

Merci de compléter l'ensemble des rubriques et de lire les notes de bas de page.

Etablissement d'inscription : Ecole Centrale de Lyon ¹
École doctorale : ED 160 EEA de Lyon dirigée par Mr Delachartre Philippe
Intitulé du doctorat : Ingénierie pour le vivant ²
Sujet de la thèse : Étude de la diversité et de la distribution des mycovirus dans le sol
Unité de recherche : Ampère ³ , dirigée par Christian Vollaire
Directeur/trice de thèse : Mr NICOL Graeme
Co-directeur/trice de thèse (le cas échéant)⁴ :
Co-directeur/trice de thèse en entreprise (le cas échéant) :

¹ A impérativement choisir dans la liste suivante : Ecole Centrale de Lyon, INSA de Lyon, Université Claude Bernard Lyon 1

² A impérativement choisir dans la liste suivante : Automatique // Electronique, Micro et Nano-électronique, Optique et Laser // Génie Electrique // Ingénierie pour le vivant Traitement du signal et de l'Image)

³ A impérativement choisir dans la liste suivante : Laboratoire Ampère, CITI, CREATIS, INL, LAGEP, LGEF

⁴ Un/une co-encadrant-e n'est pas nécessairement co-directeur/trice de thèse puisque pour remplir ce rôle, il est nécessaire d'être habilité à diriger des recherches (pour plus de précision, voir le règlement intérieur de l'ED EEA, section 3.

Collaboration(s)/partenariat(s) extérieur(s) éventuels⁵ :

Dr. Christina HAZARD has applied to the France-Berkeley Fund to support an international collaboration between our lab and that of Dr. Joanne EMERSON (Assistant Professor, University of California, Davis), who is a leading expert in soil viral ecology and viral 'omics'. The funds would support exchange trips between the two lab groups (PI's and respective PhD students) and sequencing costs for 18 metatranscriptomes derived from Mediterranean grasslands in France and USA (18 months, 2021-2023; 12K €). CH and JE are also applying for further funding to support sequencing efforts through the DOE Joint Genome Institute, Community Sequencing Program (USA). Additionally, through the established collaboration between CH and Dr. Mélanie ROY (MCF, Université Paul Sabatier) via the Labex CEBA grant 'MultiMyco', the research program will also have the opportunity to use samples taken from tropical forests in French Guiana.

Domaine et contexte scientifiques :

Fungi and other microorganisms drive soil nutrient cycling, contribute to plant productivity, and are essential for soil ecosystem function. Abundant viruses infect these diverse soil microbiota, suggesting important but largely unknown roles for viruses in soil ecosystems. In the oceans, viruses kill 20-40% of microbial cells daily, impacting global ocean food webs, carbon and nutrient cycling, aggregates and climate. Although viral impacts on terrestrial ecology have been less thoroughly investigated, our recent work, and those of others, on bacterial viruses has revealed thousands of previously unknown viral species and suggests that both host populations and abiotic factors contribute to viral distribution and dynamics, and that viruses play key roles in regulating biogeochemical cycles in soil.

Mots-clefs : Champignons, virus, sol, écosystème, biodiversité

Objectifs de la thèse :

The overall aim of this thesis is to investigate fungal viruses (mycoviruses), which are virtually unknown members of soil ecosystems. The specific objectives are to 1) determine the diversity of mycoviruses in soil ecosystems, 2) determine mycoviral distribution at the local and global scale, 3) the impact of mycoviruses on functionally important fungal guilds and 4) how mycoviral communities differ among soil fractions.

Verrous scientifiques :

Since the first description of a mycovirus in 1962, only ~200 mycoviruses have been described. The known diversity of mycoviruses is extremely small, and most prior mycoviral studies have focused on single-host model organisms, particularly plant pathogens of

⁵ Hors contrats doctoraux fléchés UMI par l'établissement, les sujets de thèse en cotutelle ne sont pas acceptés.

agriculturally important crops. In these laboratory studies, mycoviruses have shown benign, negative, or positive effects on their fungal hosts, including reduced growth, sporulation, and virulence, or increased tolerance to abiotic stress and pathogenicity. Despite this evidence for substantial mycoviral impacts on fungal ecology, the diversity and distribution of mycoviruses in complex natural communities is virtually unexplored. In particular, viruses of functionally important fungal guilds, such as plant-colonizing mycorrhizal fungi, are largely unknown, despite being recognized for their importance in ecosystem functioning. Arbuscular mycorrhizal fungi (AMF), which are the dominant mycorrhizal type in grasslands, arable fields, and many tropical forests, are ancient, obligate symbionts of 80% of the world's land plants, and they provide nutrients, drought tolerance, and pathogen protection to plants. Of the 338 currently described AMF species, only three have been reported to harbor viruses. Given that a recent metatranscriptomic study revealed hundreds of previously unknown mycoviruses in grassland soils, these culture-based efforts are clearly undersampling true mycoviral diversity. Overall, previous work on mycoviruses suggests that they play important roles in the top-down control of fungi in nature, yet mycoviral diversity is vastly undersampled, limiting our understanding of viral contributions to fungal ecology.

Contributions originales attendues :

This research program will substantially expand known mycoviral diversity, likely revealing thousands of previously unknown mycoviruses. The prevalence of mycoviruses will be assessed, particularly those of important fungal guilds, such as mycorrhizal fungi, in soil ecosystems, further elucidating the significance of viral predation on fungal communities essential to plant productivity and biogeochemical cycling. Furthermore, how viral communities differ among soil fractions, the extent to which we can successfully enrich for mycoviruses in AMF-colonized root and/or spore fractions relative to total soil, and how RNA viral communities compare across ecologically similar environments at both local and global scales will be determined.

Programme de recherche et démarche scientifique proposée :

This research program consists of sampling soil from representative Mediterranean grasslands, arable fields and tropical forests from sites that are part of ongoing research efforts in France, French Guiana and the USA. A metatranscriptomics and bioinformatics approach will be utilized for the recovery of fungal viral sequences, and statistical analyses will be used to compare mycoviral communities across ecosystems and soil fractions.

Nearly all known mycoviruses have RNA genomes. The recovery of RNA viral communities from three fractions (total soil, AMF-colonized plant roots, and purified AMF spore communities) of soil will be performed. Based on routine approaches, plant root colonization by AMF will be measured in the lab, and colonized roots will be used for downstream analyses. AMF spores will be isolated from the soil samples using wet-sieving,

decanting, and sucrose gradient centrifugation. To complement the viral analyses, fungi in each soil and root sample and AMF spores will be taxonomically identified using DNA sequencing approaches (internal transcribed spacer (ITS) and SSU AMF-specific amplicon sequencing) using our in-house high-throughput sequencing platform. Each of the soil fractions will be subjected to metatranscriptomics. Briefly, total soil RNA will be extracted and rRNA depleted, and cDNA libraries will be prepared and multiplexed on 150 bp paired-end Illumina NovaSeq runs to generate 10 Gbp of sequence per metatranscriptome. A bioinformatics pipeline for identifying RNA viral populations will be developed, utilizing the ECL Newton server. Metatranscriptomic assemblies will be mined for RNA-dependent RNA polymerases (RdRps, which are representative RNA viral hallmark genes). Viral taxonomy and host prediction will be estimated by phylogenetic placement of RdRps, and abundances will be estimated via transcript read mapping to assembled population sequences and to a developed curated RNA viral reference dataset. Routine ecological analyses, such as Principal Coordinates Analyses (PCoA), Network analyses, Mantel tests, and PERMANOVA will be applied to the relative abundance data to compare RNA viral communities across samples.

Encadrement scientifique :

- **Description du comité d'encadrement :** [à compléter avec le rôle dans l'encadrement scientifique (en termes de compétences scientifiques, etc.) et le pourcentage d'implication du directeur de thèse ⁶ et des autres membres du comité⁷]

Nom Prénom	Labo / Equipe	Compétences scientifiques	Taux d'encadrement %
Mr NICOL Graeme	Ampère Bio-Ingénierie	Écologie moléculaire, microbiologie des sols	50
Mme HAZARD Christina	Ampère Bio-Ingénierie	Écologie moléculaire, microbiologie des sols	50

- Le comité d'évaluation de l'HCERES ayant demandé à l'école doctorale de limiter la taille du comité d'encadrement à deux membres (directeur de thèse compris), il est impératif de ne proposer des comités d'encadrement de taille plus importante que si cela est absolument nécessaire⁸ et **de le justifier soigneusement.**
- **Intégration au sein du (ou des) laboratoire(s) (Département/Equipe(s) impliquée(s)) (pourcentage du temps travail au sein de ce ou ces laboratoire(s)) :**

100% Ampère Laboratoire

⁶ Le directeur de thèse doit être un HdR rattaché à l'ED EEA ou en passe de le devenir avant juin de l'année en cours ou bénéficier d'une dérogation du Conseil Scientifique lors du dépôt du sujet de thèse.

⁷ Dans le cas d'un comité d'encadrement réparti sur plusieurs établissements, la plus grande partie de l'encadrement est effectuée par des membres de l'établissement. Si l'encadrement de la thèse implique des membres hors de l'ED EEA, la part de l'encadrement des membres ED doit être très supérieure à 50%.

⁸ Un certain nombre de commissions type CNU ne reconnaissent un co-encadrement qu'au-delà d'un certain pourcentage. Souvent l'encadrement est considéré comme effectif si > 30%.

Financement de la thèse : Contrat doctoral de l'établissement d'inscription

Profil du candidat recherché (prérequis) :

- Educational background in microbiology, virology or mycology
- Research experience in microbiology, virology or mycology
- A desire to learn bioinformatics
- Highly motivated
- Team player

Objectifs de valorisation des travaux de recherche :

- Publications in peer-reviewed scientific journals
- Presentations at international conferences

Compétences qui seront développées au cours du doctorat :

- Fundamental skills in fungal and viral ecology (isolation, identification and characterization of fungi and viruses)
- Field sampling & sample processing (soil and plant roots)
- Molecular techniques (DNA and RNA extraction, library construction and sequencing)
- Bioinformatics (pipeline development and database construction)
- Community ecology statistical analyses (R software and packages)
- Manuscript preparation for publication

Perspectives professionnelles après le doctorat :

- Academic career (Postdoctoral Researcher)
- Industry, Private and Government sector (Virologist, Bioinformatician, Molecular techniques Technician)

Références bibliographiques sur le sujet de thèse :

Research articles:

- 1) Lee S, Sieradzki ET, Nicolas AM, Walker RL, Firestone MK, Hazard C, Nicol GW. 2020. Methane-derived carbon flow through host-virus trophic networks in soil. Preprint at <https://doi.org/10.1101/2020.12.16.423115>
- 2) Hazard C, Gosling P, Van der Gast C, Mitchell DT, Doohan FM, Bending GD. 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. *ISME Journal* 7:498-508. <https://doi.org/10.1038/ismej.2012.127>
- 3) Starr EP, Nuccio EE, Pett-Ridge J, Banfield JF, Firestone MK. 2019. Metatranscriptomic reconstruction reveals RNA viruses with the potential to shape carbon cycling in soil. *PNAS* 116:25900-25908. <https://doi.org/10.1073/pnas.1908291116>

Review articles:

- 1) Emerson JB. 2019. Soil viruses: A new hope. *mSystems* 4:e00120-19. <https://doi.org/10.1128/mSystems.00120-19>
- 2) Sutela S, Poimala A, Vainio EJ. 2019. Viruses of fungi and oomycetes in the soil environment. *FEMS Microbiology Ecology* 95:fiz119. <https://doi.org/10.1093/femsec/fiz119>
- 3) Chen M, Arato M, Borghi L, Nouri E, Reinhardt D. 2018. Beneficial services of arbuscular mycorrhizal fungi – from ecology to application. *Frontiers in Plant Science* 9:1270. <https://doi.org/10.3389/fpls.2018.01270>