1. General Project Information

Project name : **Viral and Bacterial Communities and Interactions in Western Siberian *Sphagnum*-peatlands – Mukhrino Area**

Acronym : **VIRPEAT**

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Site of research : **Mukhrino Field Station** (partner number 20)

Duration of the project : 45 man-days (3*15), from June 30th to July 14th

Date INTERACT Call : 01/08/2013-30/09/2013 for access visits taking place between October 2013 and October 2014

2.Background

Peat accumulation and decomposition processes are usually explained by the strong constraints of the environment, limiting microbial activities. Nevertheless, a couple of works on the net carbon (C) balance demonstrated that the accumulation would not exceed 10-15 % of the net production of the ecosystem (Francez & Vasander 1995 ; Roulet et al. 2007). Processes involved in the decomposition of organic matter and C-cycling are not well-known, despite the number of studies coupling the analysis of microbial communities and enzymatic activities (Artz 2009). Only a very few works focused on the C-trajectories through microbial communities and food-webs in peatlands (Artz et al. 2008). Description and identification of micro-organisms are well-documented, at both taxonomic and molecular (Dedysh et a. 2006 ; Kulichevskaya et al. 2008) levels, but their contribution to C-cycling in the peat are virtually unknown except for methanotrophic bacteria (Morris *et al.* 2002)

Viruses are recognized as the most dominant biological particles in the biosphere. (Kimura et al 2008).They play a key role in genetic and ecological transfers at the ecosystem level through their interactions with bacteria and others micro-organisms of the microbial loop. **To the best of our knowledge, the influence of viruses on bacterial communities abundance and diversity, and their capacity to shunt the microbial loop have never been studied in *Sphagnum*-dominated peatlands.**

Interactions between viruses and their prokaryotic host are mediated by two main viral replication cycles (Maurice et al. 2010). The lytic cycle corresponds to the direct production of viral particles, leading directly to the lysis of the host. On the contrary, the lysogenic cycle corresponds to the integration of the viral genome (called prophage) in the host genome. As a consequence, viral genome is maintained and replicated along with the host genome (the lysogen), until the lysis is induced (Paul 2008). Factors controlling viral cycles (lytic or lysogenic) and the induction of the lysogenic cycle are not easy to identify. Lysis would be favoured in productive ecosystems (Payet & Suttle 2013) while lysogenesis would be more variable, less predictive, and linked to the fluctuating physiology of prokaryotes (Maurice et al. 2010). Due to environmental heterogeneity, lysogeny is believed to be favoured in soils (Williamson et al. 2007 ; Kimura et al. 2008), even if little experimental data are available to support this hypothesis. **The same result is expected in peat, as a consequence of potential host-cells' heterogeneity of abundance and activity.**

Sphagnum-dominated peatlands cover a small area of the terrestrial ecosystems (less than 5%) but store about 1/3 of the whole stock of C in the soils. Then, since the beginning of

the Holocene, they significantly contribute to the control and the regulation of the atmosphere C-cycle (Smith et al. 2004). They are expected to significantly respond to climate changes in the coming decades and centuries. **There is therefore a need to understand how potential feedback processes are going to impact climate changes, and more precisely phage/prokaryotes interactions.**

For these reasons, it is of crucial importance i) to explore how viral and bacterial interactions will be balanced, by monitoring spatio-temporal variations of their communities and ii) to experimentally quantify how climate change could affect these interactions and the C-trajectories through microbial food-webs and C-turnover.

VIRPEAT has strong links with other research programmes and projects that are based on both field studies and laboratory experiments (EC2CO_INSU CNRS France, 2011-2012). In the medium term, the proposed study would be developed in other boreal countries, such as Finland and Canada in which we have scientific contacts (Prof. Harri Vasander in the University of Helsinki and Prof. Line Rochefort, University of Laval and CEN.

3. Objectives

The 2 mains objectives of VIRPEAT are :

i) to analyse the spatio-temporal dynamics of viruses and bacteria in a range of western Siberian *Sphagnum*-dominated peatlands. This analyse includes time-scale considerations (daily and seasonal variations of communities) and spatial patterns (i.e. lawns, hummocks, hollows / fens, bogs) corresponding to typical states of peatland ecological successions ;

We hypothesize that spatio-temporal patterns will influence viral and bacterial abundances, and the balance of the ratio 'lytic/lysogenic cycle'.

ii) to quantify the impact of an increased temperature and a change in water content, using the field experiment system (Open Top Chamber) recently installed in Mukhrino Research Station.

We hypothesize that a moderate increase of temperature and changes in water content would significantly impact viral and bacterial abundances and the proportion of lysogens. These parameters are expected to affect the sink-source functioning and C-cycling in peatlands by modifying the microbial loop.

VIRPEAT project will be very relevant for both EU and Siberian partners : the opportunity for the French team is of a high value : i) to perform experimental research on a very representative model of pristine *Sphagnum*-dominated peatlands and ii) to extend the preliminary conclusions obtain in a temperate site. Our Russian hosts will gain i) an expertise on the study of viral and bacterial communities and ii) the possibility to compare western Siberian *Sphagnum*-peatlands to other peatlands from the North Hemisphere. However, VIRPEAT will also enable both teams to start collaborations with specialists from different disciplinary topics.

4. Research methods and material, ethical issues, permits

The research will be conducted in collaboration with colleagues from the Yugra State University and Mukhrino Field Station. The station manages *Sphagnum*-dominated sites equipped with piezometers and sensors which monitor the physical-chemistry of both water and moss-carpets. Some of these parameters are needed to link abiotic features to bacterial and viral abundances. An Open Top Chamber(OTC) device is also available to monitor the long-term effect of a moderate warming on the ecosystem functioning.

Location of sampling sites

Viral and Bacterial communities will be studied in a range of characteristic fens and bogs in the Mukhrino Field Station area, in order to analyse the variations within and across *Sphagnum*-dominated peatlands. To obtain data in a context of climate change, samples will also be collected in the OTC device in order to highlight the effect of increasing temperature and moisture changes on viral and bacterial communities.

Sampling and counting viruses and bacteria

Water will be sampled directly from the *Sphagnum* carpet using a modified syringe in order to avoid peatlands disturbance. Samples will be filtered at 125 µm, 10µm and 3 µm to remove as much organic matter as possible. Viral particles and bacteria will be counted using flow-cytometry.

Estimation of the pourcentage of lysogens in bacterial communities

Water samples will be collected and filtered in fen and bog stations. Viral production and lysis of lysogens will be induced using Mitomycin C. The proportion of lysogens will be estimated as the difference between control samples (without mitomycin) and induced samples. This is monitored using flow-cytometry.

5. Implementation : time-table, budget, distribution of work specific logistics needs

Sampling and preliminary analysis will be made at the beginning of July 2014 (2^{nd}-12^{th} July 2014), corresponding approximately to the maximum of bacterial activity. As we want to sample a panel of sites around Mukhrino Field Station and different stations inside each site (lawn, hollow and hummock, fen *vs* bog), 3 persons (ECOBIO) will be needed to achieve the plan. A total of 9500 € (see below) is required to cover the travelling and logistic costs.

TIME-TABLE	**June**	**July 2014**												
	30	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M
Travel Rennes- Khanty-Mansiysk	**X**														
Travel to Mukhrino Station		**X**													
Sampling, measurements, 1st analysis			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**		
Travel back to Khanty-Mansiysk														**X**	
Travel Khanty- Mansiysk/Rennes															**X**

WORKPACKAGES	**Dates**	**Man-days**	**Distribution of work**
FRANCEZ André-Jean *Assistant Professor*	30/06/2014 to14/07/2014	15 (11 + 4 days for travel)	Coordination/ Choice of stations/Vegetation/Sampling/
BALLAUD Flore *PhD Student*	30/06/2014 to14/07/2014	15 (11 + 4 days for travel	Sampling and analysis of viral communities
QUAISER Achim *Assistant Professor*	30/06/2014 to14/07/2014	15 (11 + 4 days for travel	Sampling and analysis of bacterial communities
	TOTAL M-D 2014:	**45**	

COSTS (€) TRAVELLING	**Visa**	**Rennes-Paris**	**Paris-Moscow**	**Moscow/Khanty-Mansiysk**	**Total/ person**	
FRANCEZ André-Jean	300	200	700	1000	2200	
BALLAUD Flore	300	200	700	1000	2200	***Total travelling:***
QUAISER Achim	300	200	700	1000	2200	**6600 €**

	Flasks	Filtration material	Other consom.	***Total logistic costs***	**TOTAL PROJECT:**
LOGISTIC	550	1800	550	**2900 €**	**9500 €**

6. Expected results and data access

VIRPEAT project will allow us to generate a reliable source of comparison for the first results obtained on a single peatland in France (Ballaud et al. 2013), and to obtain more data on the impact of climate change on viral and bacterial abundances and interactions. Results will be analysed along with data from different peatlands in the Northern Hemisphere. Therefore, they are expected to be published in high impact factor journals. We also plan to give free access to raw collected data by the time they will be published. Applicants for the VIRPEAT project already work within the 'Service National d'Observation TOURBIERES' and proved to be efficient in the diffusion of data. VIRPEAT is also expected to be the starting-point of further collaborations in the future.

7. Key literature

Relevant publications of the user group

Gogo S., **Francez A.-J.,** Laggoun-Défarge F. et al. **(Accepted).** Simultaneous estimation of actual litter enzymatic catalysis and respiration rates with a simple model of C dynamics in *Sphagnum*-dominated peatlands. ***Ecosystems.***

Limpens J., Granath G., Aerts R., Heijmans M. M. P. D., Sheppard L.J., Bragazza L., Williams B.L., Rydin H., Bubier J., Moore T., Rochefort L., Mitchell E.A.D., Buttler A., van den Berg L.J.L., Gunnarsson U., **Francez A-J.** et al.. **(2012)**. Glasshouse vs field experiments: do they yield ecologically similar results for assessing N impacts on peat mosses ? ***New Phytol*** 195: 408–418

Francez A.-J ., Pinay G., Josselin N., Williams B.L. **(2011)**. Nitrogen addition triggers denitrification and affects carbon cycling in *Sphagnum magellanicum* peat. ***Biogeochemistry*** *106:435–441*

Vandenkoornhuyse P., Dufresne A ., **Quaiser A.** , Gouesbet G., Binet F . , **Francez A.-J.** et al. **(2010)**. Integration of molecular functions at the ecosystem level : breakthroughs and future goals of environmental genomics and post-genomics. ***Ecology Letters*** 13: 776–791.

Artz R.E., Chapman S.J., Siegenthaler A., Buttler A., Mitchell E.A.D., Bortoluzzi E., Yli-Petays M., Vasander H., **Francez A.-J**. **(2008)**. Functional microbial diversity in cutover peatlands responds to restoration and is directed by labile carbon. ***Journal of Applied Ecology*** 45: 1799-180

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Ballaud F, Dufresne A, **Francez AJ**, Colombet J, Sime-Ngando T**, Quaiser A.** (2013). Analysis of viral and bacterial abundance and diversity in a French *Sphagnu*m-dominated peatlan, by metagenomics. Oral communication, AFEM Congress, Clermont-Ferrand, France, 22-25th October 2013.

Dedysh SN, Pankratov TA, Belova SE *et al* (2006). Phylogenetic analysis and in situ identification of Bacteria community composition in an acidic *Sphagnum* peat-bog. *Appl Environ Microbiol* 72: 2110-2117.

Francez A.-J., Vasander H. (1995). Peat accumulation and peat decomposition after human disturbance in French and Finnish mires. *Acta Oecologica* 16:599-608.

Kimura M, Zhong-Jun J, Natsuko N, Susumu Asakawa (2008). Ecology of Viruses in Soils: Past, Present and Future Perspectives. *Soil Science & Plant Nutrition* 54: 1–32.

Kulichevskaya IS, Ivanova AO, Baulina OI (2008). *Sungulisphaera acidiphila* gen nov, sp nov, a non-filamentous, *Isosphaera*-like planctomycete from acidic northern wetlands. *Int J Syst Evol Microbiol* 58: 1186-1193.

Maurice, C. F., C. Bouvier, R. de Wit, Bouvier T (2010). Linking the Lytic and Lysogenic Bacteriophage Cycles to Environmental Conditions, Host Physiology and Their Variability in Coastal Lagoons . *Environmental Microbiology* 15: 2463–2475.

Morris SA, Radajewski S, Willison TW, Murrell JC (2002). Identification of the functionally active methanotroph population in a peat soil by stable-isotope probing. *Appl Environ Microbiol* 68: 1446-1453.

Paul J (2008). Prophages in Marine Bacteria: Dangerous Molecular Time Bombs or the Key to Survival in the Seas? » *The ISME Journal* 2: 579-589.

Payet, J.P., Suttle A (2013). To Kill or Not to Kill: The Balance Between Lytic and Lysogenic Viral Infection Is Driven by Trophic Status. *Limnology and Oceanography* 58: 465 474.

Roulet NTP, Lafleur M, Richard PJH, Moore TR, Humphreys ER, Bubier J 2007. Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Global Change Biology* 13:397-411

Smith L.C., MacDonald G.M., Velichko A.A. *et al*. (2004). Siberian peatlands a net carbon sink and global methane source since the early Holocene *Science* 303 : 353-356.

Williamson KE, Radosevich M, Smith DW, Wommack KE (2007). Incidence of Lysogeny Within Temperate and Extreme Soil Environments. *Environmental Microbiology* 9: 2563–2574.