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Soil water flow is a source of the plant pathogen *Pseudomonas syringae* in subalpine headwaters

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ABSTRACT

20 The air-borne plant pathogenic bacterium *Pseudomonas syringae* is ubiquitous in headwaters, snowpack and precipitation where its populations are genetically and phenotypically diverse. Here, we assessed its population dynamics during snowmelt in headwaters of the French Alps. We revealed a continuous and significant transport of *P. syringae* by these waters in which the population density is correlated with water chemistry.

25 Via *in situ* observations and laboratory experiments, we validated that *P. syringae* is effectively transported with the snow melt and rain water infiltrating through the soil of subalpine grasslands, leading to the same range of concentrations as measured in headwaters (10^2 to 10^5 CFU l⁻¹). A population structure analysis confirmed the relatedness between populations in percolated water and those above the ground (i.e. rain, leaf litter and

30 snowpack). However, the transport study in porous media suggested that water percolation could have different efficiencies for different strains of *P. syringae*. Finally, leaching of soil cores incubated for up to 4 months at 8°C showed that indigenous populations of *P. syringae* were able to survive in subalpine soil under cold temperature. This study brings to light the underestimated role of hydrological processes involved in the long distance

35 dissemination of *P. syringae*.

INTRODUCTION

In Europe, most of the river runoff is controlled by alpine streams, which drain a total land area of 1.1 million km² and discharge 3.7×10^{14} litres of water annually (Edwards et al., 40 2007). Flow regimes and chemical characteristics of alpine streams are determined by the relative contribution of snowpack melt water, rainfall, glacial ice melt and groundwater, under a climatic and geologic context (Brown et al., 2003). The role of water flow through this fluctuating environment in the ecology and dissemination of microorganisms is still poorly understood. Overall, studies of alpine watersheds have focused on describing microbial 45 community structure and function in lakes (Felip et al., 1995; Alfreider et al., 1996; Felip et al., 1999; Hortnagl et al., 2010; Newton et al., 2011). Only a few studies have addressed dynamics of microbial communities in alpine streams in response to environmental changes (Battin et al., 2004; Fierer et al., 2007), but none have addressed questions about how landscape influences the population dynamics of individual microbial species.

50 *Pseudomonas syringae* is a plant pathogen whose dissemination is linked to the water cycle (Morris et al., 2008; 2010). It is abundant in alpine and subalpine ecosystems where it is present in seasonal snow cover at high population densities in leaf litter and senescent grasses in particular (Morris et al., 2008; Monteil et al., 2012). It was estimated that leaf litter and snowpack harbor about 10^8 cells of *P. syringae* per m² of subalpine 55 meadow and those in headwater worldwide represent 10^{20} cells; a population size comparable to that estimated to be harbored by plants (Morris et al., 2010; Monteil et al., 2012). Various authors have observed highly diverse populations in these atypical habitats with a broad range of host range profiles usually associated with agricultural environments (Gardan et al., 1999; Sarkar and Guttman, 2004). The subalpine environments of *P.* 60 *syringae* constitute reservoirs of pathogenic strains (Monteil et al., 2013), and therefore the dissemination of strains via stream flow from this reservoir to regions of agricultural

production is particularly pertinent. Nevertheless, long distance transport of *P. syringae* with subalpine runoff and its population dynamics during snowmelt has not yet been explored.

Subalpine runoff in streams and rivers is driven by the drainage of snowmelt and rainfall through the soil and bedrock fractures. When the water infiltrates the soil, its chemistry may change due to different geochemical processes as a function of factors that are specific to each catchment basin—e.g. the water infiltration rate, snowpack depth, residence of water in the soil, the duration of frost or the mineral composition of the environment (Campbell *et al.*, 1995; Edwards *et al.*, 2007). In subalpine ecosystems, most melt water infiltrates into the subsurface soil environment contrary to high elevation alpine ecosystems where longer periods of soil frost are a greater impediment to infiltration (Edwards *et al.*, 2007; Williams *et al.*, 2009). Therefore, the occurrence of *P. syringae* in streams suggests that (i) most of the *P. syringae* populations from precipitation and plant tissues in meadows are transported through the soil via water infiltration and that (ii) the water chemistry of streams could be a marker of passage of *P. syringae* via the soil. Such processes, that imply the ability to survive in soil for a certain duration, have never been assessed before for *P. syringae*, nor for any plant pathogen. Most studies of this bacterium have corroborated its poor capacity to survive in soils. Crop soils in particular do not seem to have a critical role as a habitat, especially without the incorporation of diseased plant tissue (Kritzman and Zutra, 1983; McCarter *et al.*, 1983; Riffaud and Morris, 2002; Zhao *et al.*, 2002; Hollaway *et al.*, 2007; van Overbeek *et al.*, 2010). However, in alpine ecosystems, Reynolds and Ringelberg (2008) reported the successful transfer of non-indigenous *P. syringae* populations from snow to soil where the bacterium was detected up to 80 days after transfer. Similarly, Goodnow *et al.* (1990) observed high rates of viability of a strain inoculated into subalpine soil at 7.5°C. Thus, questions still remain concerning the extent of *P. syringae*'s capacity to survive in soil.

Here, we have evaluated the capacity of *P. syringae* to flow through alpine soil with snowmelt or rainwater. In a study of population dynamics in the headwaters of several

snow-fed streams in the Southern French Alps during snowmelt, we tested the hypotheses
90 that (1) headwaters continuously carry bacteria in spring and summer and (2) the bacterial
concentrations in headwaters are correlated with their hydrochemistry. We also tested the
hypothesis that (3) bacteria are transported with rainwater and snowmelt through subalpine
soils. Two approaches were used: (i) *in situ* transfer of *P. syringae* through subalpine
grasslands soils was assessed by quantifying naturally occurring populations in percolated
95 rainwater sampled from deep seepage collectors and (ii), the transfer of indigenous cells of
P. syringae and of a marked strain introduced into soil columns was monitored. In a last
step, we tested a fourth hypothesis that different *P. syringae* strains have different abilities to
transfer through the soil. We showed that soil water flow is a source of *P. syringae* in
subalpine headwaters and the presence of the bacterium seems to be associated with
100 landscape features.

RESULTS & DISCUSSION

***P. syringae* is systematically detected during snowmelt in streams of catchment basins of the French Alps**

To validate that subalpine headwaters were significant carriers of *P. syringae* during
105 snowmelt, we assessed the population dynamics of this bacterium from March to July in
2009 and 2010. *P. syringae* was systematically detected in the streams of four subalpine
catchment basins in the French Alps at concentrations of 10 to 10⁵ CFU l⁻¹ (*cf.*, an example
of a sampling site in Fig. S1). As shown in Table S1, we confirmed that *P. syringae*
populations are highly diversified in water regarding traits associated to pathogenicity, such
110 as the capacity to induce an HR on tobacco, to produce syringomycine-like toxins, to be ice
nucleation active or about the aggressiveness on cantaloupe. These results support
previous observations of *P. syringae* in river water (Morris *et al.*, 2010). Overall, statistical
analyses support the conclusion that the concentration of *P. syringae* increased over time
(Fig. 1A), while no consensus trend was observed between sites for total bacteria (Fig. 1B);

115 for which population sizes seemed to increase only for Soudane creek (Spearman's rank test, $P = 0.07$). The omnipresence of *P. syringae* and its population dynamics over time suggest a continuous flux of bacteria during the snowmelt period strongly supporting that the bacterium is transported by water discharge passing through the soil.

120 These observations allowed us to estimate the fluxes of *P. syringae* coming into a cropping area, thereby providing a unique opportunity to evaluate the potential risk of a plant pathogen in water used for the irrigation of cultivated plants. Retention basins surrounding crops have been reported to be reservoirs of *P. syringae* (Riffaud and Morris, 2002). Inoculum in these irrigation water sources was assumed to be from local runoff from cropped fields. Here, we show that the subalpine hydrological network has the potential to contribute
125 to the microbiological composition of irrigation water. Based on the mean of all population sizes of *P. syringae* found in the streams of the Upper Durance River (UDR) basin, the average population size in headwaters during snowmelt (March to July) is 2000 CFU l⁻¹. These streams converge to the UDR outlet at the Serre-Ponçon lake, regulated for hydropower production by Électricité de France (Lafaysse et al., 2011). According to river
130 flow measurements between 1950 and 2006, daily mean flow at the outlet is about 78 m³ s⁻¹ with a range of 14 to 919 m³ s⁻¹, corresponding to a daily transport of 10¹² to 10¹⁴ cells of *P. syringae* towards the Durance outlet in cropping areas.

135 ***Bacterial abundance and hydrochemical properties concur with the influence of landscape features to the population dynamics of P. syringae in surface waters***

The estimation of flux presented above does not take into account the structure and geographic variability of *P. syringae* populations in the UDR hydrological network. Consequently, after determining the population dynamics over time, we investigated the effect of the sampling location on bacterial population sizes. There was a significant effect
140 of the catchment basin on population sizes of *P. syringae* and of total bacteria (Table 1).

Pisse creek in the Ceillac catchment basin had significantly lower population sizes compared to Soudane creek in the Super Sauze catchment basin (Pair-wise Student t-tests, $P < 0.01$). Intermediate population sizes were quantified in the creeks in the two other catchment basins. *P. syringae* concentrations were positively correlated to those of total bacteria (Fig. 2), suggesting the plant pathogen was susceptible to the same processes impacting the whole bacterial community. The differences between sites could be linked to environmental variability and water source. At the spatial scale of this study, field observations do not allow us to assess the mechanisms underlying the survival rate or transport rate. However, these field observations studies are crucial to identify links between population sizes and environmental variables, and subsequently to raise hypothesis to test in future mechanistic studies. Here, we decided to investigate the association between the creek hydrochemistry and bacterial population sizes.

Hydrochemistry gives important information about water origin and history because it is the result of the dynamics between snowmelt, groundwater and glacial ice melting, within a context influenced by interactions between the climatic, geologic and biotic conditions that influence the chemicals that are likely to be solubilized into water (Ward et al., 1999; Brittain and Milner, 2001; Brown et al., 2003; Hannah et al., 2007). The few studies that have addressed the response of microbial communities in alpine streams to their environment have revealed various relationships between water dynamics, hydrochemistry and microbial population structure. For example Fierer et al. (2007) observed an increase of Proteobacteria population size when the stream water pH increased while the opposite trend was observed for Acidobacteria. Battin et al. (2004) pointed out an effect of the hydrological regime and hydrochemistry of streams on bacterial population size in alpine microbial biofilms. Here, the snow-fed streams of the different sites had statistically distinct hydrochemistry profiles in terms of electrical conductivities (EC, Table 1) and relative abundances of ions (i.e. SO_4^{2-} , Mg^{2+} , Ca^{2+} , Fig. 3). Streams of the Col de Vars, Col du Lautaret and Super Sauze catchment basins were typical of calcic bicarbonated waters,

whereas that of Ceillac (Pisse creek) was typical of calcic sulfated waters (Piper, 1944), for which the total ionic concentration (826 mg l^{-1}) and EC ($609 \mu \text{ S cm}^{-1}$) were 2–3 times higher. Other parameters were not significantly different among the streams (pH of 7.90, DOC of 3.26 mg l^{-1} and 5.6°C on average at the time of sampling, Table 1, Pair-wise Student t-tests, $P > 0.05$).

Correlations between microbial population sizes and chemical parameters showed that the concentrations of *P. syringae* in streams were inversely correlated with EC and $\text{SO}_4^{2-} / \text{Mg}^{2+}$ concentrations, but positively correlated with alkalinity (Table 2). These significant trends were also observed for total bacteria (Table 2). Indeed, high $\text{SO}_4^{2-} / \text{Mg}^{2+}$ concentrations were characteristic of Pisse creek waters which also had the lowest concentrations of *P. syringae* and total bacteria. The Pisse creek headwater starts downstream of a large lake in the Ceillac catchment basin. It is surrounded by Triassic outcrops rich in gypsum and dolomite-bearing rocks, whose dissolution might explain the high conductivities and high concentrations of SO_4^{2-} and Mg^{2+} (Meybeck, 1987; Darmody et al., 2000; de Montety et al., 2007). But high conductivities can also be the result of deeper inflow from groundwater (Ward et al., 1999). On the contrary, headwaters dominated by HCO_3^- can be indicative that the headwater chemistry is more strongly influenced by rainfall and snowmelt than by mineral dissolution (Meybeck, 1987; Campbell et al., 1995). Three hypotheses emerge from these observations: (i) paths of water flow through soils with different mineral contents filter the bacterial populations differently, (ii) chemistry has a direct impact on bacterial survival and (iii), the bacterial concentration in streams reflects the concentration in the source (e.g. snowmelt, infiltrated lake water, groundwater, ice melt). Here, the association between DOC amounts and *P. syringae* concentrations (Table 2) support all hypotheses. When water infiltrates into the soil during snowmelt or rainstorms, it can drain high amounts of DOC mainly in subsurface environments (Boyer et al., 1997; Battin et al., 2004; Williams et al., 2009). Therefore, this correlation suggests that the more snowmelt is flushing through subsurface and near-surface soil horizons, the higher the

195 bacterial loads in streams. This process would depend on microbial population sizes from leaf litter, snowpack and possibly the first horizon of soil. Future studies should focus on distinguishing the effect of the source (water origin and microbial habitat) on the microbial population dynamics in streams from that of water transport dynamics through the soil.

200 ***P. syringae* is transported by water through tens of centimeters of subalpine soil**

Our observations that *P. syringae* flowed continuously in headwaters of subalpine creeks raised questions about the source of the populations of this bacterium. In subalpine catchment basins, water flow through the soil is the main source of creek water in settings such as those used in our study (Campbell *et al.*, 1995; Edwards *et al.*, 2007; Williams *et al.*, 2009). We thus used two approaches to validate directly the transport of *P. syringae* with subsurface water flow through subalpine grassland soils. Firstly, we determined if *P. syringae* was present in the percolated water from lysimetric-mesocosms located at the Col du Lautaret. *P. syringae* was detected in 9/12 samples of seepage water which had percolated through 30 cm of grassland soil after two rainfall events in June 2010 (illustrated in Fig. S2). Population sizes from percolated samples were highly variable between samples ranging from 10 to 1.5×10^4 CFU l^{-1} (Table 3). The occurrence of *P. syringae* in the environment of Col du Lautaret (leaf litter, standing vegetation, rain and snowpack) was checked during the same year when percolated water was collected (Monteil *et al.*, 2012) (Table 3). A total of 97 strains (40 from percolated water) was collected, characterized and assigned to genetic clades as described previously (Morris *et al.*, 2010). All samples contained strains of clades TA003, 2a, and 4 in varying proportions; with the TA003 clade being the most represented (77% in percolated water and 60% in other habitats). A clustering analysis performed with the software STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003) as described previously (Morris *et al.*, 2010), based on 89 Single Nucleotide Polymorphism sites (SNPs) and 22 haplotypes revealed five clusters for which the dominant

haplotypes were detected in all substrates. These results suggest that populations successfully percolating through the soil reflect those that were dominant on the local vegetation, leaf litter and in precipitation (Fig. 4). The absence in the water of two clusters among the five determined by the structure analysis is most likely due to their population sizes being below our detection level rather than the result of filtering by the soil. This corroborates the notion that the diversity of *P. syringae* in soil water depends on its diversity locally in litter and vegetation whereas this latter diversity can vary among sites (Monteil *et al.*, 2012).

In a second approach to demonstrate the transport of *P. syringae* in soil, we allowed a suspension of *P. syringae* to percolate through undisturbed subalpine soil core monoliths and then estimated its rate of transfer through the soil. Soil core monoliths were extracted at the three sites for which headwaters were studied (illustrated in Fig. S3). Strain TA022 of *P. syringae* was used in these experiments because it belongs to the clade most frequently isolated in percolated water of the lysimetric-mesocosms at the Col of Lautaret (TA003 clade). The mutant line TA022-rif was selected for resistance to 200 mg l⁻¹ of rifamycin. Resistance to rifamycin was used as a marker to track inoculated populations because its rate of occurrence in indigenous bacterial populations from the soil core monoliths was below the detection threshold for the microbiological analyses used in this study. TA022-rif was systematically detected in the leachates from the soil core monoliths. We estimated its transfer rate (C/C_0) to be between 12% and 17% (Table 4) by taking into account the growth of the inoculum during the percolation period relative to the sizes of the leaching populations and of the initial inoculum. There was no significant effect of the origin of the soil core monolith on this rate (KW, $P > 0.05$), even though it is likely that the transfer rate could vary with soil properties and the *P. syringae* strain. Indeed, the porous medium structure of soil is heterogeneous (e.g. organic and mineral contents, grain size, grain composition, porosity and water content). Moreover, the thickness and the physical and chemical structure of these soil horizons vary according to geology, topography, climate and vegetation cover

(Abu-Ashour *et al.*, 1994; Ginn *et al.*, 2002). Evaluating the intraspecific variability of transfer rate relative to the physical-chemical structure of soil and land occupation is challenging.

250 Nevertheless, as percolating soil water gives rise to headwaters, knowing this transfer rate would lead to an understanding of how each climatic-hydrologic-geologic context and vegetation diversity in subalpine environments could select different genetic lines of *P. syringae*, some of them potentially being new pathotypes, and contribute to their transport towards cropped regions (Monteil *et al.*, 2013).

255 ***P. syringae's cell surface charge and transfer rate through homogeneous porous media vary among strains***

We showed that *P. syringae* is transported along soil core monoliths and that the populations in percolated water of field lysimeters are genetically diversified. Regarding the different frequencies of clades in the percolated water, we wondered if strains inside the *P. syringae* species complex are able to transfer at the same rate. We thus addressed this

260 issue by estimating the variation of transfer rate within the *P. syringae* complex in saturated, homogeneous and sterile porous media. The porous medium is represented of soil in general but not necessarily of the specific soils from the field sites studied here. In addition to the physical properties of the soil, biological factors can also influence the transfer rate of

265 bacteria. Electrical surface properties, hydrophobicity or production of EPS in particular can affect bacterial transfer rates in soil (Hermansson, 1999; Ginn *et al.*, 2002; Jacobs *et al.*, 2007). In the primary stage of bacterial attachment to surfaces, the phenomenon of attraction / repulsion between the porous medium and the overall charge of the cells (ζ potential) is one of the overriding factors that determines the adhesion of bacteria

270 (Hermansson, 1999). Therefore, to choose strains for this study, we determined the ζ potentials of 17 strains representing different clades of the *P. syringae* complex (Morris *et al.*, 2010) (Table 5). This property has not been described for *P. syringae*. To approximate real chemical conditions observed in creek samples, ζ potentials of these strains were

measured in a solution of CaCl_2 at $100 \mu\text{S cm}^{-1}$, pH 7. Means of ζ potentials ranged from
275 -27 mV to -11 mV (Table 5, P values of pair-wise t-tests are shown in Table S2) and did not
depend on the phylogenetic situation of the strain. As van der Mei and Busscher (2001) and
Jacobs et al. (2007) reported, we also observed high standard deviations (SD) within the
population of cells of a single strain for each electrophoretic measurement (assessed for
several thousands of cells). These high SD (up to 9mV for some strains), testify to the
280 presence of subpopulations inside the clonal population itself as reported for other bacteria
(van der Mei and Busscher, 2001). Bacterial cell surface heterogeneity (including
hydrophobicity) is widespread in microorganisms and allows them to adapt to various
environmental conditions (van der Mei and Busscher, 2012). Such observations raise
questions about the factors controlling this heterogeneity and its role in the transport of *P.*
285 *syringae* through the soil and for its attachment to surfaces in general.

The transfer of *P. syringae* in porous media was estimated for seven strains (CC94,
SZ-30, UB-246, B728a, USA102, DC3000 and TA022) under the same conditions as those
used for measuring ζ potential. The strains were chosen to represent the full range of ζ
potentials. The rate of transfer across the porous medium (Fontainebleau sand, Table S4)
290 varied significantly among strains (Table 5). For example, only 6% of the injected population
of DC3000 leached through the sandy columns whereas for USA102 this rate was 10 times
higher. However, the transfer rate could not be predicted from the ζ potential (Pearson
correlation test, $P < 0.05$) as previously observed for some bacterial species (Gannon et al.,
1991). The combination of other cell surface properties such as hydrophobicity, presence of
295 flagella, and exopolysaccharides might have an overriding effect on transfer (Hermansson,
1999; Ginn et al., 2002; Jacobs et al., 2007). Nevertheless, even if the experimental
conditions used here are a simplified representation of real soil and do not take into account
all the environmental and biological variability, our results suggest that the rate of passage of
P. syringae through soil could vary considerably among strains. The role of genetic and

300 phenotypic variability of *P. syringae* in the survival and transport through the soil remains to
be explored in future studies. It would permit identification of the selection pressures applied
by such a process on the plant pathogen populations outside of agricultural contexts. By
extrapolation, if the population structure of *P. syringae* varies with local conditions such as
vegetation, then this could lead to marked differences in abundance of this bacterium in
305 headwaters depending on the transfer capacity of the main genotypes in the population.

Under cold conditions P. syringae survives in soil for several months

Our results demonstrated that soil water flow was a source of *P. syringae* in creeks. It
raised the question of the ability of *P. syringae* to survive in the soil when water is not
flowing. To assess survival of bacteria in the soils of the three sites represented in this
310 study, soil core monoliths collected at the three sites were incubated at 8°C for 4 months.
This is representative of the temperature average of subalpine soils measured in others
studies during the snowmelt period (Reichstein *et al.*, 2000; Margesin *et al.*, 2009; Clement
et al., 2012; Saccone *et al.*, 2013). After incubation, soil core monoliths were saturated with
distilled water and the microbial population sizes in the percolating water were determined.
315 The soil core monoliths from the Ceillac site retained three times more distilled water than
the other monoliths, indicating that they were drier (see Table S3 for soil core
characteristics). As shown in Table 4, the total volume of water that was taken up by and
then flowed through the column caused the percolation of 10⁹ bacteria. Populations of
naturally-occurring *P. syringae* were detected in the leached water of 7 of the 9 columns at
320 concentrations from 225 to 5.4 × 10⁴ CFU l⁻¹. The concentrations of *P. syringae* detected in
the leachates corresponded to the same order of magnitude of population sizes frequently
observed in pristine mountain waters of this and in previous studies (Morris *et al.*, 2010).
Thus, a certain fraction of the *P. syringae* population survived for at least 4 months in these
soils and was readily leached as soon as water flow began.

325 This raises questions about which soil zone was at the origin of these populations:
the rhizosphere, the soil particules or the thick upper layer of organic matter and leaf litter
(Fig. S3A and B). Interestingly, in the few leachates where *P. syringae* was not detected,
the soil core monoliths had numerous tunnels from the passage of worms and a strong
anoxic odor. This was most likely due to the decomposition of small animals such as
330 invertebrates. These results offer new insight to the life history of *P. syringae* by suggesting
the ability of autochthonous populations of *P. syringae* to be competitive in the face of the
microbial communities of grassland soils at this range of temperature and humidity. Yet, the
presence of *P. syringae* in grassland soils contrasts with its rare occurrence in crop soils.
Different explanations are possible. Grassland soils are aerated and present numerous
335 aggregates rich in organic matter and with less dense roots systems. The temperature is
colder due to the altitude and there is a high level of humidity during snowmelt, close to or
greater than field capacity (Clement *et al.*, 2012). These soil conditions are quite different
from those for cropped fields where soils are usually lower in organic matter, are subjected
to higher temperatures, and have lower volumetric water contents.

340 CONCLUSION

The vectors of dissemination of plant pathogens contributes to gene flow within and
between populations resulting in a biogeographic structuration of their metapopulation. In
the case of long distance dissemination, the pathogen can be spread to regions well-beyond
where it is endemic. Spread of plant pathogens with air currents and with the commercial
345 distribution of infected plant materials are the means of long-range dissemination that have
been the most well-studied. Here we have demonstrated that *P. syringae*, well-known to be
effectively disseminated by these processes, is also transported with water flow as rain run-
off or snow melt infiltrated through the soil and into headwaters of rivers. Therefore, *P.*
syringae can potentially be transported through hundreds of km from headwaters in the
350 Southern French Alps at the highest altitudes studied here, to the main river outlet in

Southeastern France toward the Mediterranean Sea. This process is likely to be continuous along the river network with soil water flows coming from the entire watershed to enrich *P. syringae* populations of a river along its course.

The new insight into the dissemination of *P. syringae* provided by this work raises novel questions about how the population structure downstream in the irrigation network (e.g. retention basins, groundwater and river water) is influenced by (i) populations in alpine prairies, and (ii), those of crops and non agricultural environments across the river network to a larger extent. Many alpine regions are managed either for recreational activities, as summer grazing prairies or for erosion control, for example. Future studies could estimate the effect of spatial patterns of management practices on the structure of the autochthonous populations of *P. syringae* and the abundance of genetic lines that are crop pathogens. Their eventual fate in downstream irrigation networks could be evaluated in terms of local and regional geophysical and geochemical contexts and in terms of their survival rates. Our results also point to the need to re-evaluate the potential of *P. syringae* to survive in soil, a potential that has seemingly been underestimated in the ecology of this bacterium, as well as in the covering leaf litter and grasses (Monteil *et al.*, 2012). The current understanding of this potential may have been influenced by the soil conditions addressed in former studies and by a dense microbial background population that could have obscured the low population levels of *P. syringae*.

Our work also raises questions about the perimeter of agro-ecosystems. Landscapes on regional and continental scales can contribute to hydrological processes that shape the life history of pathogens such as *P. syringae* and that link cropped fields to habitats considered to be relatively more “natural” or “wild”. This is in line with the current notion of hydroecology / ecohydrology developed over the past 10 years and that overlaps Earth and Environmental sciences with Biology and Ecology. It has been applied mostly to benthic macroinvertebrate communities, fish populations, and algal communities in subalpine streams (Hieber *et al.*, 2001; Brown *et al.*, 2003; Hannah *et al.*, 2007; Milner *et al.*,

2009) to predict the response of freshwater biota and ecosystems to variation of abiotic factors over a range of spatial and temporal scales (see review by Hannah *et al.* (2004)).

380 However, it is clearly pertinent to plant pathogens disseminated by the water cycle whereby the pathogens encounter a multitude of physical-chemical and biotic environments with varying degrees of anthropogenic inputs. Furthermore, a broadened scale of study is likely to be pertinent to other plant pathogens such as *Phytophthora* spp, *Erwinia* sp., *Pectobacterium* sp. and *Fusarium* sp., for which their importance in non-agricultural

385 substrates has been observed (Franc, 1988; Cother and Gilbert, 1990; Palmero *et al.*, 2011; Hansen *et al.*, 2012). The complexity of environments encountered by plant pathogens that move from non-agricultural to agricultural habitats argues for hybrid studies in microbial ecology that integrate a range of disciplines (e.g. hydrology, geology, climatology, plant ecology) at spatial scales beyond the current perimeters of host environment as proposed

390 for human pathogens (Constantin de Magny *et al.*, 2008).

EXPERIMENTAL PROCEDURES

Sampling sites

The field study was carried out between March 2009 and June 2010 in four catchment

395 basins where land was mown but not cultivated. These sites are situated in the Upper Durance Basin (UDR) (3580 km²) located in the Southern French Alps, which supplies water for crop irrigation in Southeastern France (Lafaysse *et al.*, 2011). Sites were chosen to be near the tree line, with similar snowpack dynamics (e.g. snow depth, thawing cycles). The GPS coordinates and altitudes of the catchment basins (Ceillac, Col de Vars, Super Sauze,

400 and Col du Lautaret) are presented in Table 6. Each of these basins is associated with a snow-fed stream (Rithral streams according the classification by Brown *et al.* (2003)): Pisse creek, Riou Mounal creek, Soudane creek and Roche Noire creek respectively. Sites are characterized as open meadows (forbs, grasses and legumes) that are surrounded by

patches of forests (Larches, Mountain and Arolla pines). The mean minimum temperature of
405 the coldest month is -3°C and the mean maximum of the warmest month is 30°C , except for
the Col du Lautaret site for which means are 4°C colder. Soils have a sandy-loam texture
originating from a mixture dominated by calc-schists with eolian material.

Population dynamics of *P. syringae* in headwaters

Sampling and processing of water samples. The water of the four snow-fed streams, was
410 sampled several times during the snowmelt period at the source and at 1 to 2 km
downstream (a total of 60 samples). Water was collected into sterile sampling bags and
kept on ice in a cooler, transported to the laboratory and processed on the following day.
Population sizes of total culturable bacteria and *P. syringae* per liter of water were
determined as described below.

415 **Chemical analyses.** The stream temperature, electrical conductivity and pH were
measured at the time of sampling (Consort C561, UK, reference temperature at 25°C ,
accuracy $\pm 0.01^{\circ}\text{C}$, $\pm 1 \mu\text{S cm}^{-1}$, ± 0.01 pH unit). For chemical analyses, bicarbonate
concentrations were also determined for the crude samples by Gran titration (Gran, 1952).
About 150 ml of filtrate from each water sample were stored in polyethylene bottles in the
420 dark at 4°C until chemical analyses could be accomplished for the major ions (Mg^{2+} , Ca^{2+} ,
 Na^{+} , K^{+} , Cl^{-} , PO_4^{2-} , SO_4^{2-} , NO_3^{-}) and about 30 ml were stored in the same conditions in
brown glass bottles, stabilized with mercuric chloride, for DOC analysis. The concentrations
of the dissolved anionic and cationic species were determined by ion chromatography
(Dionex DX-120, Sunnyvale, CA, USA). The detection limits of the analyzed ions were 0.01
425 mg l^{-1} . Chemical features of water were characterized through the construction of trilinear
Piper diagrams (Piper, 1944) using the DIAGRAMMES software version 5.1 (developed by
R. Simler, Avignon, <http://www.lha.univ-avignon.fr>). For DOC analysis, we used a Bioritech
TOC 700 analyzer (Bioritech, Guyancourt, France) that eliminates total dissolved mineral
carbon by acid dissolution and then quantifies the CO_2 released during oxidation during

430 heating. Accuracy of this measurement was 0.05 mg l⁻¹. Absence of chemical contamination was checked by processing distilled water with the same material.

Detection of *P. syringae* in water percolated through subalpine soils *in situ*

A field plot of 6 lysimetric mesocosms was set up in 2007 at the Col du Lautaret subalpine station described above (Station Alpine Joseph Fourier, 2050 m a.s.l., GPS
435 coordinates in Table 6). Each lysimetric mesocosm consisted of a stainless steel cylinder (40 × 25-cm Ø) containing an intact 30-cm deep soil core with its associated vegetation originating from two local subalpine grasslands (3 replicates each) (see supplementary Fig. S2). The soil cores were composed on average of 50% sand, 30% clay, 15% organic carbon and had a pH of 7.5. The bottom 10-cm of each lysimetric mesocosm was separated
440 from the soil monolith by a metal grid and a filter so that percolating water could be collected in this empty volume. Seepage water was pumped from the lysimetric-mesocosms using a portable peristaltic vacuum system (VK-lite, UMS, Munich, Germany) with sterilized materials in June 2010 after two rain events. At the time of these events, all snow had melted and lysimetric mesocosms had been drained. In addition, the occurrence of *P.*
445 *syringae* on the leaf litter and vegetation next to the lysimetric mesocosms and in rainfall water on 16 June 2010 was determined. Samples were kept on ice in a cooler until processing. Population sizes of total culturable bacteria and *P. syringae* per gram of plant material or per liter of percolated water and rainwater were determined as described below. The pH and electrical conductivity (EC) of the water were measured as described above. A
450 set of 48 *P. syringae*-like strains selected randomly among the population percolated through the soil was put in collection as described in Monteil *et al.* (2012) for further characterization.

Transport of *P. syringae* through unperturbed soil core monoliths

Sampling and processing of soil core monoliths. Soil core monoliths were collected in
455 subalpine meadows close to headwaters of three catchment basins among the four basins

studied here: at Super Sauze, Col du Lautaret and Ceillac (illustrations of the monoliths are shown in Fig. S3). The first two sites were sampled on 4 April 2011 and the third site on 8 April 2011. At the time of sampling, the ground was covered with about 70 to 130 cm of snow that had begun melting. In spring, these meadows are dominated by herbaceous
 460 plants (Saccone *et al.*, 2013). Three core monoliths of soil per site (20 cm long and 10 cm in diameter) were sampled using PVC coring tubes. The soil core monoliths were transported in a cooler and kept for four months at 8°C until processing.

Simulation of rainfall and inoculation of soil core monoliths with traceable bacteria.

Transport of *P. syringae* through the soil core monoliths was determined by simulating
 465 rainfall containing 10^8 CFU l⁻¹ of a mutant line of strain TA022 naturally resistant to rifamycin (referred to here as TA-022-rif; the origin of this strain is described below) in sterile distilled water. Rain was simulated at a rate of 20 mm h⁻¹ for 90 min to obtain a minimum of 400 ml of effluent from each column. Once the percolation of water had finished, the bacterial transfer rate (C/C_0) was determined by quantifying the bacterial concentration as described
 470 above in the effluent (C) and in the initial inoculum after the transfer period (C_0). C_0 and C take into account the multiplication rate during the experiment and the total volume used for percolation. A scheme of the experimental design is provided in supplementary Fig. S4.

Before inoculation of the core monoliths with strain TA-022-rif, the size of indigenous
 475 populations of *P. syringae* that could be leached from the soil core was evaluated by saturating the column and by simulating a rainfall using sterile distilled water at the same rate as indicated above. Once the flow ended, the leachates were immediately processed to determine the bacterial concentrations.

Estimation of *P. syringae* transport in homogeneous porous media

The transfer of *P. syringae* in a homogenous porous medium was estimated by
 480 measuring the relative effluent concentration of a pure strain continuously injected in saturated Plexiglas columns (15 cm long, 5 cm in diameter) filled with sterilized

Fontainebleau sand (properties given in supplementary Table S5). Each column was sterilized with alcohol, filled with 480 g of cleaned sand that was autoclaved twice at 125°C for 45 minutes at 1 bar pressure and then dried at 105°C for 24 h between each cycle. The columns were first rinsed and saturated with a sterile solution of 0.06 g l⁻¹ CaCl₂. Then a bacterial suspension at 10⁷ CFU ml⁻¹ was injected at 0.6 ml min⁻¹ for 30 minutes. After injection of the bacterial suspension, the CaCl₂ solution at the concentration used for the initial rinse was injected for 13.5 h at the same flow rate as for the bacterial suspension. Leachates were collected in sterile bottles at the bottom of the column. Concentrations of *P. syringae* were determined in the inoculum prior to injection (C₀), in the inoculum at the end of the 14 h injection and leaching period (C), in the suspension containing the sterile sand and CaCl₂ solution (as negative control), and in the leachates. The rate of transfer of *P. syringae* was the ratio between C and C₀ corrected by the recovery volume to determine the transfer rate and the multiplication rate of the inoculum. For each strain, transfer was measured in three independent experiments. The absence of contamination due to the equipment used was also verified. Methods for quantification of bacterial populations are described below.

Bacterial strains and culture conditions

The reference strains used to estimate *P. syringae* transport in porous media represented most of the genomic groups defined by Morris *et al.* (2010) (Table 5). Strains were analyzed in aqueous suspensions. These suspensions were prepared from cultures grown at room temperature for 48 h on King's medium B (KB) (King *et al.*, 1954) initiated from stock cultures stored in glycerol at -80 °C. Each suspension was prepared with either sterile distilled water or with a sterile CaCl₂ solution (pH 7.5) to represent either mineral content of rain or subalpine stream chemistry. The concentration of CaCl₂ that was used in these experiments (0.06 g l⁻¹) was equivalent to a conductivity of about 100 μS cm⁻¹. The bacterial concentrations were adjusted with a spectrophotometer to 10⁷ cells ml⁻¹. For the

strain TA-022, spontaneous mutants were isolated on KBC that were resistant to rifampicin at 200 mg l⁻¹.

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510 **Characterization of ζ potential**

The ζ potential of the 17 strains of *P. syringae* described above was determined. According to the extended DLVO theory (Hermansson, 1999) that describes the interactions between charged surfaces in solution, interactions between cells and the porous medium strongly depend on the electric charge of bacterial cells under given conditions and is defined by its ζ potential. This potential is characterized by measuring electrophoretic mobility of a suspension in response to an electric field. The ζ potential of each strain for each concentration of CaCl_2 was determined for suspensions of 10^7 CFU ml^{-1} according to the theory of Smoluchowski (Elimelech *et al.*, 2000). Each measurement was performed with a Zetasizer Nano (Malvern Instruments Ltd., Malvern, UK). Several measurements were performed per strain and per condition.

Quantification of *P. syringae* and total bacterial populations in water

Treatment of the various samples obtained in this study depended on their nature. The size of bacterial populations in headwaters and leachates obtained from all the soil columns was determined by concentrating the liquid by a factor of 200 via filtration across sterile nitrocellulose membranes (0.22 μm pore diameter) before dilution plating. Aliquots of the same samples were dilution plated on general media (10% tryptone soja agar) to enumerate total bacteria, and on selective media (KBC of Mohan and Schaad (1987) as described previously (Morris *et al.*, 2008). The detection limit was 55 CFU l^{-1} for total bacteria and 5 CFU l^{-1} for *P. syringae* (This discrepancy in detection level arose because the volume plated for the detection of *P. syringae* was 10 x greater than that used for the detection of total cells). The bacterial concentrations of the pure culture inocula and those of the percolation columns of sand were determined by dilution plating on KB medium or KB containing 200 mg l^{-1} of rifamycin for the TA-22 strain. After inoculation, all media were kept at room temperature and colonies were counted after 48 and 96 h of incubation. To verify the identities of *P. syringae*-like colonies, a minimum of 30 fluorescent colonies per

treatment were checked for the absence of cytochrome C oxidase as described by Morris et al. (2008).

Genotyping of *P. syringae* strains

The identity of *P. syringae*-like strains was further determined by sequencing the *cts* housekeeping gene with primers described by Sarkar and Guttman (2004) and Morris et al. (2008). Strains were affiliated to a clade described by Morris et al. (2010), whose work showed that phylogenetic trees based on *cts* sequences were congruent with those based on the whole MLST analysis described in Sarkar and Guttman (2004).

Clustering analysis

Genetic similarity of strains from leaf litter, percolated water, snow and rain from the Col du Lautaret (Table 3) was determined by identifying clusters based on the genetic relatedness of *cts* sequences (89 SNPs in a total of 421 bp). Estimation of panmictic clusters (*k*) and strain membership to clusters were assessed with the program STRUCTURE version 2.3 (Pritchard et al., 2000; Falush et al., 2003) with the same settings described by Morris et al. (2010). We checked the congruency of the results several times and assigned strains to a cluster according a threshold of membership coefficient of $q > 0.9$.

Statistical analyzes

Statistical analyses were performed with R software version 2.15.1 (R Core Team, 2012). Means were compared either by Student's t-tests or ANOVA tests. If the underlying assumptions of the tests were not satisfied (and in particular the assumptions of normal distribution and homogeneity of variance), they were compared with the Mann Withney U-test (MWU) or the Kruskal-Wallis rank sum test (KW) with a Bonferroni correction. Correlations were determined by building a linear model and then by testing their significance by both Pearson's method and Spearman's method because the linearity of the correlation between variables is not known (Zar 1984).

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745 **FIGURES AND TABLES LEGENDS**

Fig. 1 Dynamics of *P. syringae* (**A**) and total culturable bacteria (**B**) as a function of time (number of Julien days starting on January 1st). Three of the sampling sites are represented: (i) Ceillac (Pisse creek data are represented by ● and a solid line), (ii) Col de Vars (Riou Mounal creek data are represented by ■ and a dashed line) and (iii), Super Sauze (Soudane creek data are represented by △ and a dotted line). Significance of the correlations was estimated by both Pearson's method (P) and Spearman's method (S) Coefficients and the associated probability (in superscript) are indicated for each variable and site.

Fig. 2 Concentrations of *P. syringae* in relation to total culturable bacteria in all samples pooled (n=57, $y=0.97x-2.81$, Pearson's $r = 0.75$, $P < 0.001$)

Fig. 3 Chemical features of streams sampled in the Southern French Alps represented as the ionic concentration relative to the total ionic concentration (mg l^{-1}) on the trilinear Piper diagram (Piper, 1944) (**A**). The diagram is composed of two triangles representing cations and anions, and one rhombus representing global ionic composition. A Principal-Component Analysis (PCA) followed by a Monte-Carlo test (999 replicates) on a Linear Discriminant Analysis (LDA) confirmed that chemical dissimilarities were higher between creeks than within ($P < 0.001$) (**B**).

Fig. 4 Population structure of *P. syringae* over the study area obtained with STRUCTURE (Pritchard *et al.*, 2000; Falush, 2009). The computation was performed with *cts* sequence polymorphism (97 sequences of 421bp and 89 SNPs). Frequency of strains belonging to each *k* cluster was estimated according to the origin of the sample: above ground surface substrates including snowpack and leaf litter (hatched bars) or water that had percolated through the soil (grey bars).

Table 1 Means and standard errors of electrical conductivities, pH and population sizes of total culturable bacteria and *P. syringae* in streams. For catchment basin, means were

calculated with the samples from the two locations because there was no significant effect of sampling location on any of the variables (ANOVA, $P < 0.05$). Mean values associated with the same letter are not significantly different (multiple MWU, $P < 0.05$).

Variable	ANOVA F -statistic ^a		Catchment basins and their creeks ^c			
	Creek zone ^b	Site	Ceillac (<i>Pisse</i>)	Col de Vars (<i>Riou Mounal</i>)	Super Sauze (<i>Soudane</i>)	Col du Lautaret (<i>Roche Noire</i>)
Population densities log(CFU l⁻¹)						
Total bacteria (n=57)	0.64 ^{ns}	12.11 ^{***}	5.67 ± 0.10 ^c	6.46 ± 0.16 ^{ab}	6.75 ± 0.13 ^a	6.13 ± 0.20 ^{abc}
<i>P. syringae</i> (n=57)	0.12 ^{ns}	4.96 ^{**}	2.73 ± 0.12 ^{bc}	3.30 ± 0.29 ^{ab}	3.78 ± 0.15 ^a	3.16 ± 0.07 ^b
Physico-chemical parameters						
EC (µS cm ⁻¹) (n=56)	0.42 ^{ns}	40.79 ^{***}	609 ± 44 ^a	216 ± 7 ^c	324 ± 15 ^b	143 ± 20 ^d
pH (n=56)	1.78 ^{ns}	0.28 ^{ns}	7.91 ± 0.05 ^a	7.89 ± 0.04 ^a	7.89 ± 0.06 ^a	7.79 ± 0.05 ^a
DOC (mg l ⁻¹) (n=51)	0.40 ^{ns}	0.87 ^{ns}	3.56 ± 1.05 ^a	3.19 ± 0.68 ^a	5.28 ± 1.18 ^a	3.18 ± 1.40 ^a

^a ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

^b Two zones of each creek were sampled for three sites; they were separated by more than 1 km and 400 m of altitude

^c Catchment basin names are in regular font and the associated creek is in italics

Table 2 Correlations between bacterial concentrations (log (CFU l⁻¹)) and water chemistry pooled for all samples

Variable	<i>Rho</i> of Spearman sum rank test ^a		
	<i>P. syringae</i>	Total bacteria	EC
EC	-0.33*	-0.46***	
pH	0.14 ^{ns}	0.13 ^{ns}	-0.08 ^{ns}
DOC	0.32 ^{ns}	0.38 ^{ns}	-0.04 ^{ns}
SO ₄ ²⁻	-0.30*	-0.49***	0.87***
HCO ₃ ⁻	0.39**	0.56***	-0.83***
Cl ⁻	0.33*	0.33*	-0.75***
NO ₃ ⁻	-0.05 ^{ns}	-0.10 ^{ns}	-0.55***
Ca ²⁺	-0.19 ^{ns}	-0.29*	0.52***
Mg ²⁺	-0.41**	-0.64***	0.68***
Na ⁺	0.38**	0.43**	-0.85***
K ⁺	0.24 ^{ns}	0.33*	-0.82***

^a ns denotes $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Correlations were determined with 51 samples, except for EC and pH (n=56).

780 **Table 3** Microbial characteristics of the percolates from lysimetric mesocosms, of rainfall
 water and of the surrounding plant material in grasslands from the Col du Lautaret
 experimental site. Data from this study were completed with those from Monteil *et al.* (2012)
 from November 2009 and March 2010 (precipitation, snowpack and vegetation) for which
 each sample was collected according to similar procedures. Each population size in plant
 785 material represents three independent bulks from a surface of 30 m². The pH and electrical
 conductivity of the percolate and precipitation samples were also determined. Means are
 associated with their standard errors. The *cts* sequences of strains characterized in this
 study can be downloaded from <http://www.pamdb.org>.

Season*, Sample	Population sizes log (CFU l ⁻¹ of water) or log (CFU g ⁻¹ of plant material)		Sequenced strains	pH	EC
	Total bacteria	<i>P. syringae</i>			
June 2010					
<i>Percolated water</i>	7.67 ± 0.17	1.96 ± 0.32	CLA41-47, 366-373, 396-403, 416-423, 446-450, 452-453, 462, 468, 469	6.16 ± 0.10	56 ± 6
<i>Rainfall</i>	3.92	2.52	CLA492 to CLA501, 509	6.34	4
<i>Leaf litter</i>	7.97 ± 0.07	3.39 ± 1.08	/	/	/
<i>Grassland plants</i>	6.78 ± 0.12	4.19 ± 0.77	/	/	/
March 2010					

<i>Leaf litter</i>	7.96 ± 0.16	5.38 ± 0.47	CLA275, 282, 283, 284, 291, 296, 302, 303, 311, 316, 318, 319, 321, 322, 325, 326, 330	/	/
<i>Snowpack</i>	8.79 ± 0.35	6.78 ± 0.08	CLA175, 189, 192, 200, 201, 206, 207, 209, 212, 214, 217, 219, 235, 239, 251, 252, 257	/	/
November 2009					
<i>Snowfall</i>	5.92	1.90	CLA71 to CLA82	6.60	14

* Two different experiments of three replicates were conducted on 10 and 18 June 2010. Means were determined on 12 samples of percolated water. The presence of the bacterium was determined in one of the rainfall events of 16 June 2010.

Table 4 Population sizes of microorganisms extracted from the soil core monoliths. Microorganisms were quantified in the percolated water after the simulation of a rain with sterile distilled water above the unperturbed soil core monoliths stored 4 months at 10°C (3 sites, 3 replicate core monoliths per site). One week later, microorganisms were quantified after a simulated rain containing 10^7 CFU ml⁻¹ of the marked strain TA022 was applied to the same core monoliths. To account for the multiplication rate during the 14 hours of the transfer experiment, TA022 populations were quantified in the suspension used for the rain simulation at the end of the experiment. Mean values in the same row associated with the same letter are not significantly different (Pair-wise Student t-tests, $P < 0.05$).

Concentration log (CFU l ⁻¹)	Site		
	Ceillac	Col du Lautaret	Super Sauze
Indigenous bacteria in percolated water ^a			
Total bacteria	9.35 ± 0.08 ^A	8.96 ± 0.08 ^B	9.65 ± 0.12 ^A
<i>P. syringae</i>	2.35 ± 0.73 ^A	2.14 ± 0.64 ^A	3.85 ± 0.51 ^A
Inoculum 14h old (strain TA022)	8.45 ± 0.04 ^A	8.81 ± 0.02 ^A	8.41 ± 0.21 ^A
Bacteria in percolated water ^b			
Total bacteria	8.68 ± 0.04 ^A	8.81 ± 0.27 ^A	8.97 ± 0.25 ^A
TA022	7.56 ± 0.06 ^A	7.73 ± 0.26 ^A	7.38 ± 0.14 ^A

Transfer rate of TA022	0.12 ± 0.03 ^A	0.17 ± 0.09 ^A	0.12 ± 0.06 ^A
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^a before the introduction of strain TA022

^b after the simulation of rainfall at the surface of the soil core monoliths

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Table 5 Origin and characteristics of *P. syringae* strains used in this study, their cell surface charges (ζ potential) and transfer rates through porous media. Transfer rates (C/C_0) were measured for strains representing the range of ζ potential values by using suspensions with the same characteristics (concentration of bacteria and ions) used for the determination of ζ potential. Means have been calculated with 15 values for ζ potential and 9 replicates for transfer rates in porous media. Means associated with the same letter are not significantly different (Pair-wise Student t-tests, $P < 0.05$). “NA” means Not Analyzed.

Strain	ζ potential (mV) ^f	C/C_0	Clade ^a	HR ^b	INA ^c	AGR ^d	Source	Ref ^e
USA-032	-10.70 ± 2.24	NA	USA-032	+	+	-	Water	(1)
B728a	-10.81 ± 1.41	0.385 ± 0.074 ^{BC}	Groupe 2b	+	+	+	Bean	(2)
CC0440	-14.96 ± 2.26	NA	Groupe 2a	+	+	+	Cantaloup	(3)
Cit7	-16.08 ± 1.47	NA	Cit7	+	+	+	Orange	(4)
CC0663	-18.18 ± 1.23	NA	TA002	-	+	-	<i>Primula</i> sp.	(5)
USA-102	-18.18 ± 2.02	0.593 ± 0.062 ^A	USA-102	+	+	+	Water	(1)
CC94	-18.32 ± 1.85	0.231 ± 0.022 ^C	Groupe 2b	+	+	+	Cantaloup	(3)
UB-370	-18.40 ± 1.57	NA	UB-370	+	+	-	Water	(1)
TA022	-19.50 ± 1.97	0.424 ± 0.070 ^{AB}	TA003	+	+	+	Water	(1)
1448a	-21.42 ± 1.89	NA	Groupe 3	+	+	+	Bean	(6)
CC1513	-21.76 ± 1.43	NA	Groupe 4	+	+	-	<i>Hutchinsia alpina</i>	(5)
UB-246	-23.54 ± 1.53	0.304 ± 0.067 ^{BC}	UB-246	-	-	-	Water	(1)
DC3000	-23.58 ± 1.74	0.058 ± 0.007 ^D	Groupe 1	+	+	-	Tomato	(7)
SZ-030	-24.16 ± 1.40	0.293 ± 0.025 ^{BC}	SZ-030	-	+	-	Water, crops	(3)

TA006	-24.34 ± 1.27	NA	TA006	+	-	-	Water	(1)
TA020	-26.02 ± 1.44	NA	TA020	+	-	-	Water	(1)
CC1524	-26.76 ± 1.42	NA	CC1524	-	-	-	Water, biofilms	(5)

^a according to the phylogeny described by Morris *et al.* (2010)

^b hypersensitivity reaction on tobacco

^c ice nucleation activity : positive strains induce freezing at -8°C or warmer in a test of 10⁶ cells (Morris *et al.*, 2008)

^d aggressiveness on cantaloupe : positive strains induce symptoms on more than half of the cotyledons tested (Monteil *et al.*, 2012)

^e (1) Morris *et al.* (2010); (2) Hirano *et al.* (1997); (3) Morris *et al.* (2000); (4) Lindow (1985) ; (5) Morris *et al.* (2008) ; (6) Taylor *et al.* (1996); (7) Cuppels (1986)

^f Probabilities associated with paired comparisons according to the Student t-test are given in Table S3. ζ potentials were measured for all the strains in a suspension at 100 $\mu\text{S cm}^{-1}$ (CaCl_2 0.06 g l⁻¹) and at a density of 10⁷ CFU ml⁻¹

Table 6 GPS coordinates of sampling sites. Catchment basins are in regular font and the name of the creeks are in italics.

	Substrate	Altitude (m) ^a	Latitude	Longitude	Nb of samples
I.1.1. Sites					
Ceillac (<i>Pisse creek</i>)	Soil	2200	44°38'05"N	06°47'22"E	/
	Water	1690	44°38'54"N	06°47'33"E	9
		2200*	44°38'10"N	06°47'19"E	9
Col de Vars, (<i>Riou Mounal creek</i>)	Water	1880	44°31'49"N	06°42'38"E	7
		2100*	44°32'11"N	06°42'11"E	11
Col du Lautaret (<i>Roche Noire creek</i>)	Soil	2100	45°02'12"N	06°24'08"E	/
	Water	2000*	45°02'37"N	06°24'53"E	3
Super Sauze (<i>Soudane creek</i>)	Soil	2000	44°20'40"N	06°41'57"E	/
	Water	1600	44°21'14"N	06°42'05"E	11
		2000*	44°20'38"N	06°42'00"E	7

^a Sites associated with a star correspond to the river source

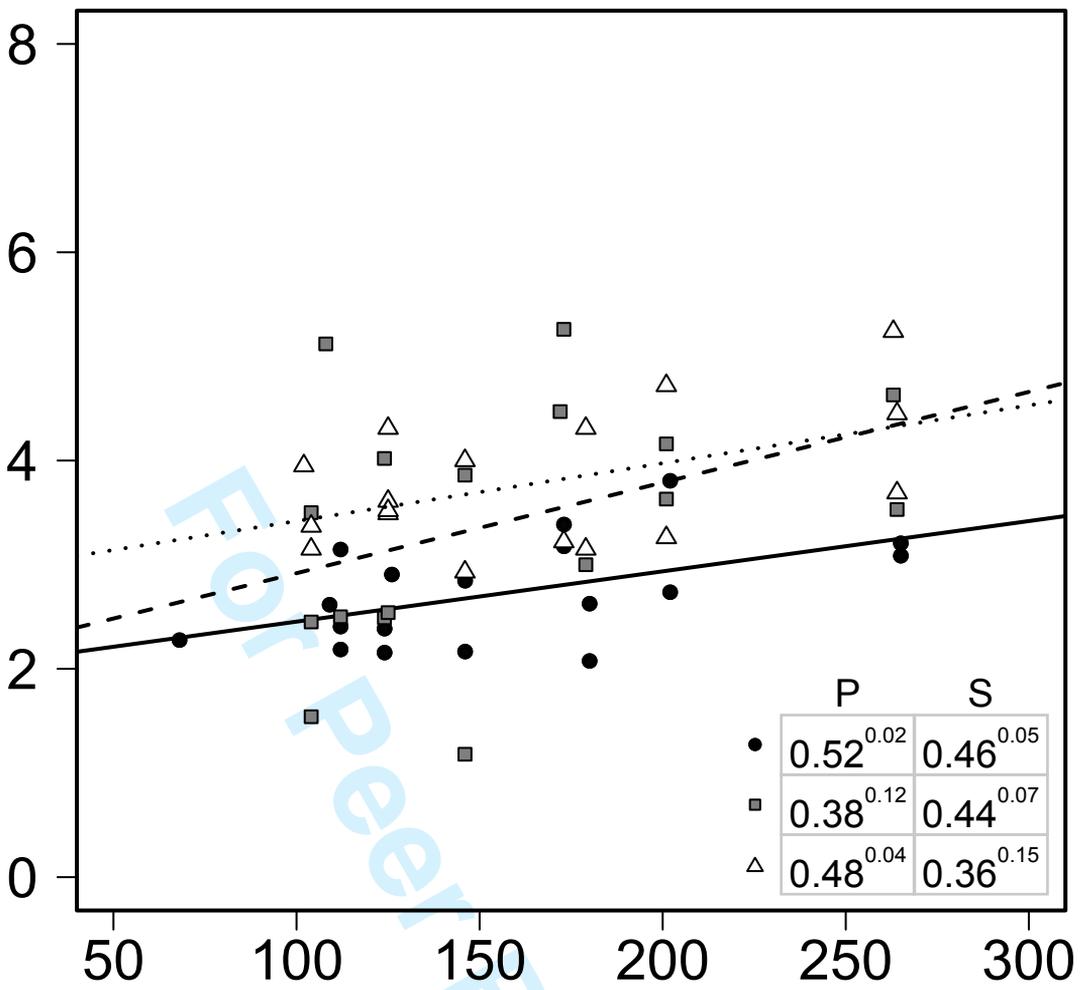
815 **Supplementary information****Table S1 to S4** See separate files

Fig. S1 Headwater of the Riou Mounal creek (Col de Vars site) on 26 May 2010 illustrating the exfiltration of the water from the soil. General view (**A**) and zoom on the sampling point
820 (**B**).

Fig. S2 Set-up of lysimetric mesocosms located in the experimental area of the Joseph Fourier Alpine Station at the Col du Lautaret, France. Experimental plots with the meteorological stations and the lysimetric mesocosms (**A**), a typical lysimetric mesocosm with its soil core and vegetation (**B**) and the peristaltic system for pumping the percolated
825 water from the bottom reservoir of the lysimetric mesocosms (**C**).

Fig. S3 Sampling of soil core monoliths . A pit of 2m² under the snow was dug, then the first 30 cm of soil was removed almost down to the bedrock. The soil was characterized by a distinctive aggregated and lumpy structure with the presence of a dense root system (**A**) and a dense accumulation of organic matter at the surface (**B**). Each PVC tube was then
830 placed on the surface (**C**) and the bordered soil was removed gradually by bevelling and gently pressing until the tube was filled (**D**). Once the column was ready, it was closed and isolated at both ends to avoid exchanges with the outside (**E**).

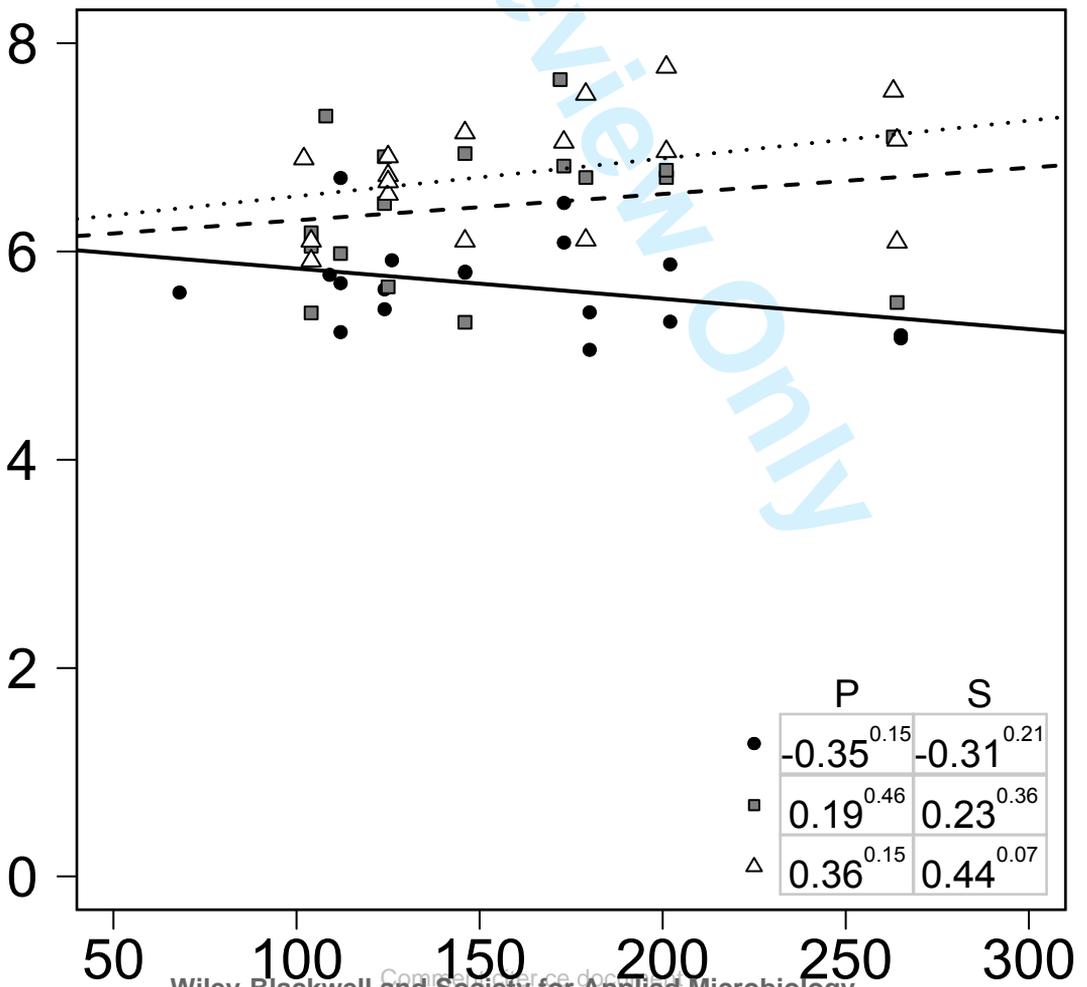
Fig. S4 Scheme of the system for rain simulation over soil core monoliths. The bacterial suspension was pumped while under agitation and then sterile distilled water was applied.
835 Eight sterile capillaries distributed drops on the core surface. Percolated water was collected in a closed and sterile bottle for further quantification.

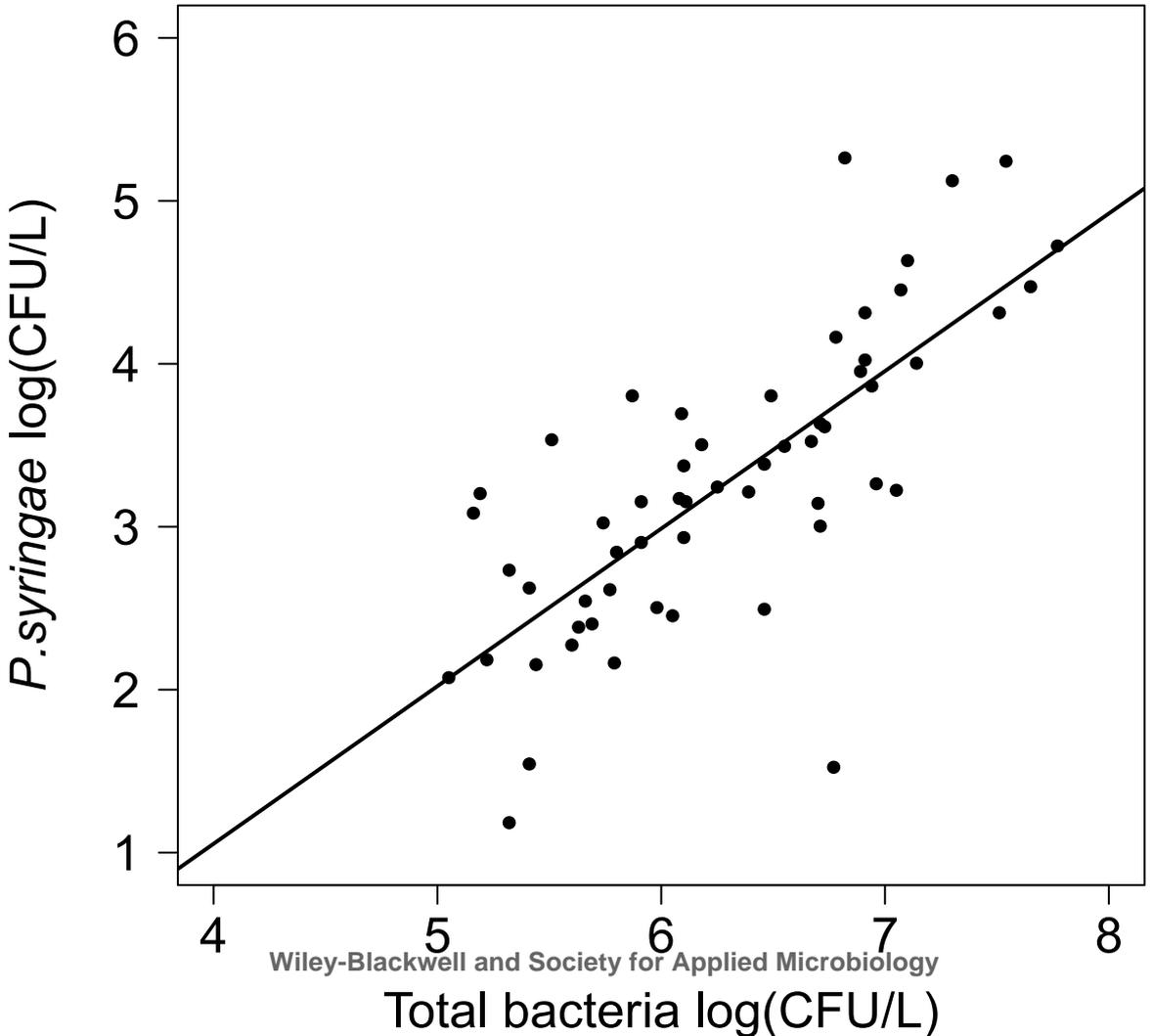
A*P. syringae* log(CFU/L)

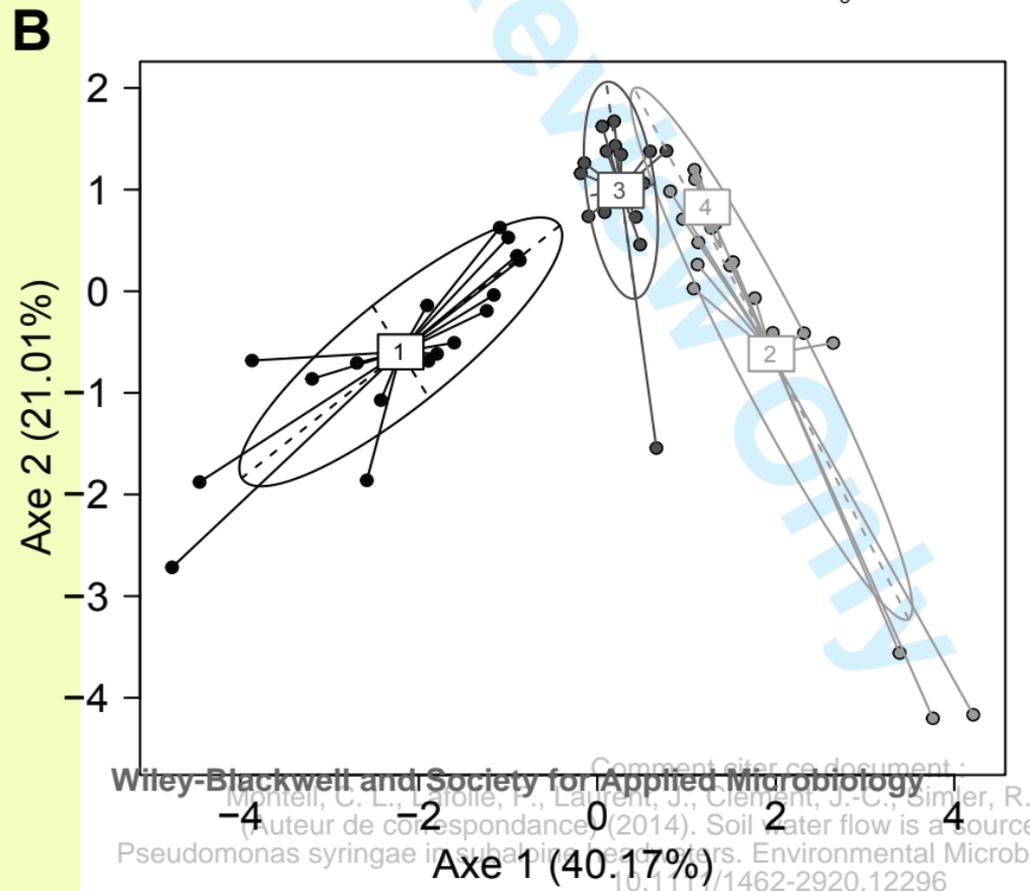
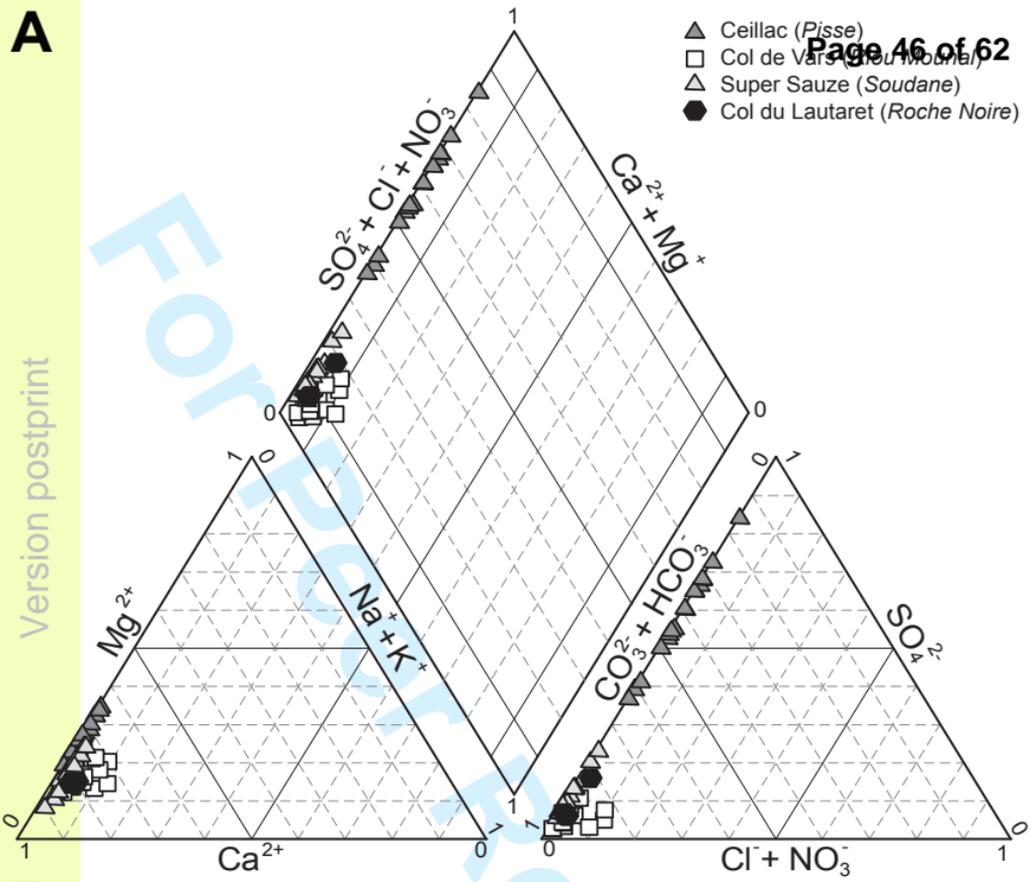
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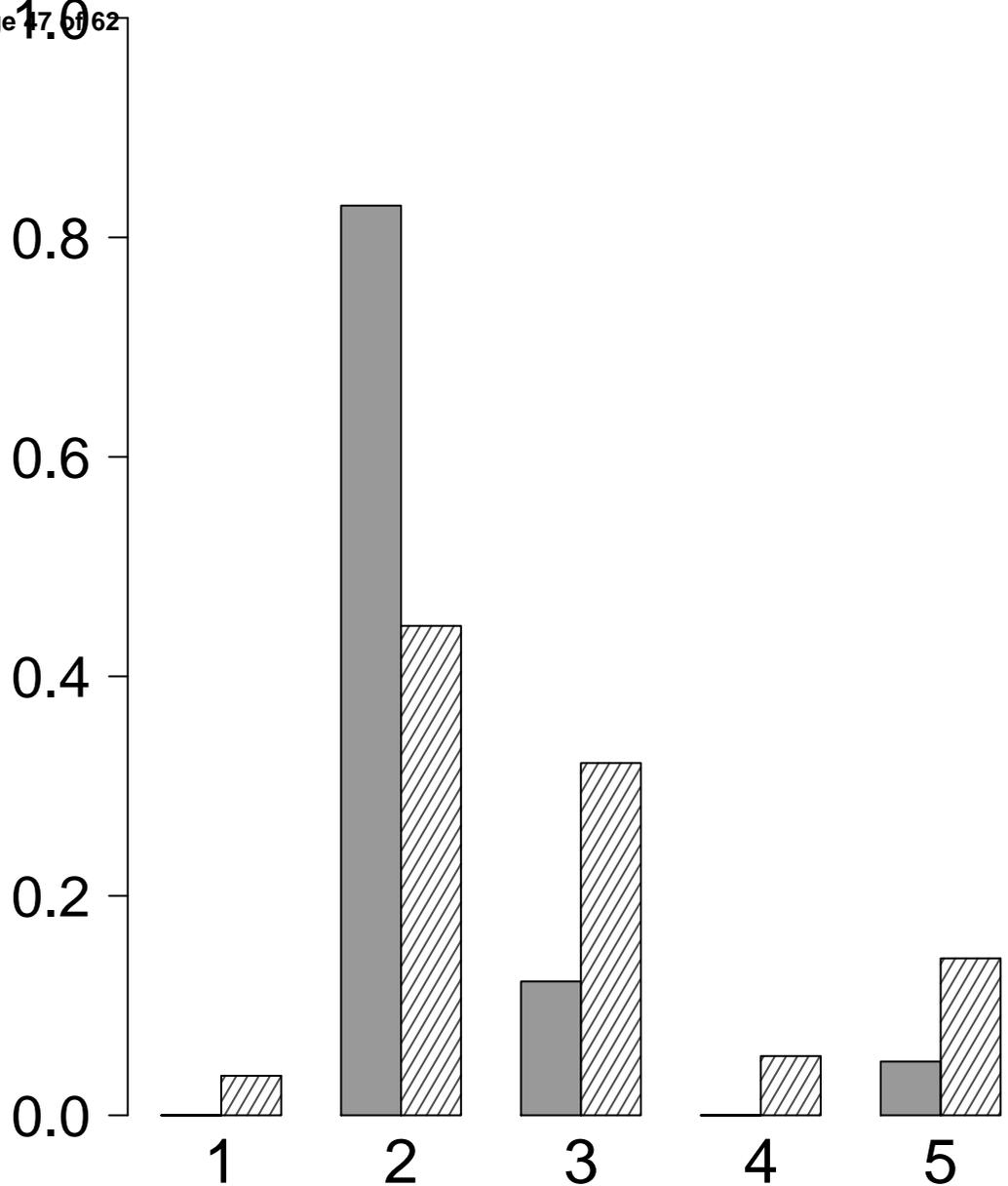
Total bacteria log(CFU/L)







Frequency of strains



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Cluster

A**B**





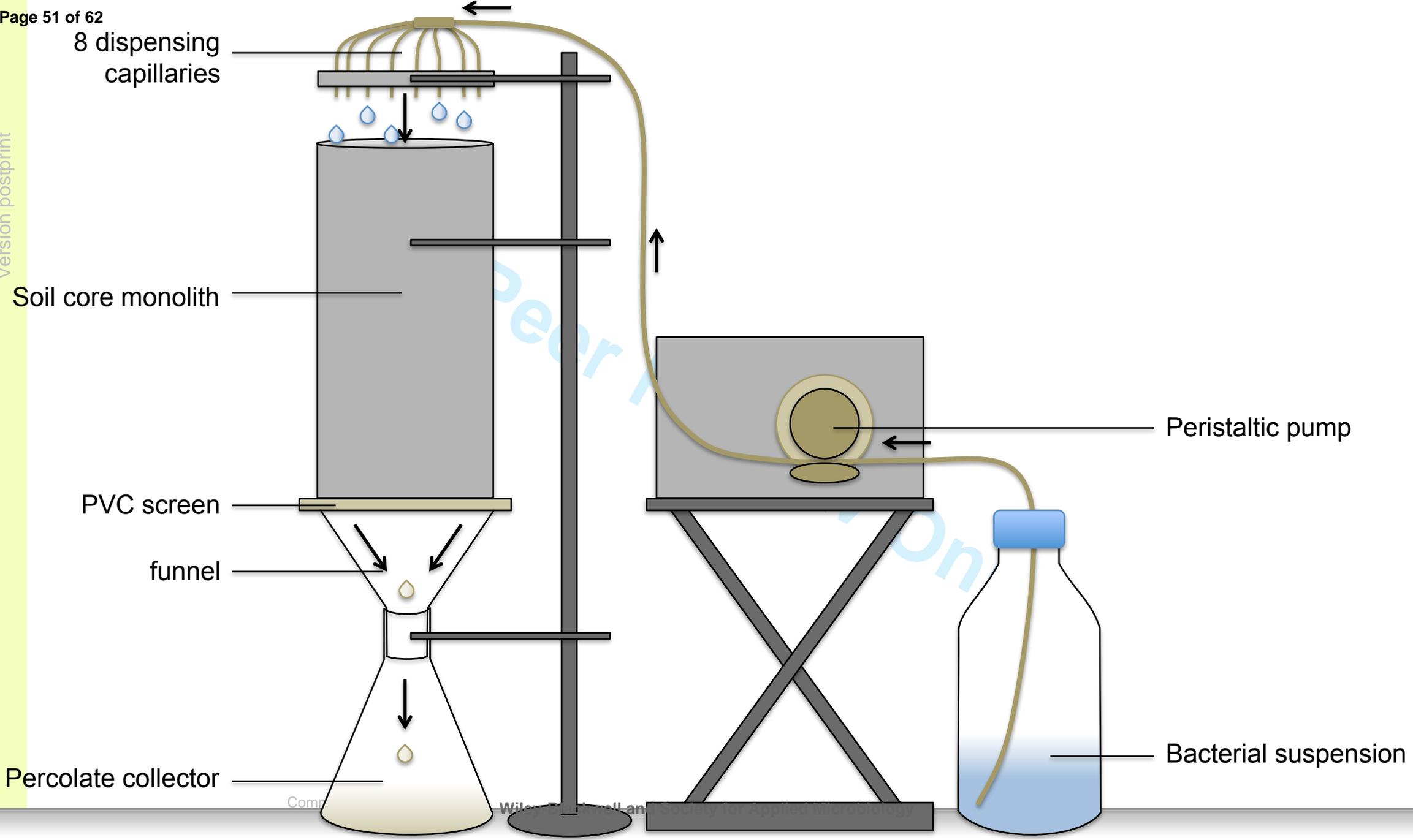


Table S1 Frequencies of *P. syringae* strains in different phenotypic classes from water at three sites in 2 based on the same protocol of Monteil et al. (2012).

Site ^a	Sample name	Date	Frequency of strains in each class (%)					
			HR ^{+b}	INA ^{+c}	INA ^{3d}	AGR ^{+e}	AGR ^{>1f}	SYR ^{+g}
Ceillac (<i>Piise creek</i>)								
	CEW1	10/3/2009	96	100	45	14	0	24
	CEW2	04/23/2009	89	94	53	20	0	20
	CEW3	04/23/2009	86	90	43	24	15	67
	CEW7	05/27/2009	100	100	35	25	16	26
	CEW8	05/27/2009	76	95	38	43	14	52
	CEW9	06/23/2009	33	71	9	62	50	5
	CEW10	06/23/2009	44	94	28	55	39	20
Col de Vars (<i>Riou Mounal creek</i>)								
	CVW1	04/15/2009	33	95	19	19	5	45
	CVW2	04/15/2009	76	100	29	43	19	76
	CVW5	05/27/2009	65	90	40	25	10	70
	CVW7	06/23/2009	NA	NA	NA	80	80	85
	CVW8	06/23/2009	27	91	35	64	50	18
Super Sauze (<i>Soudane creek</i>)								
	SZW1	04/13/2009	95	100	30	20	5	30
	SZW2	04/13/2009	80	95	40	25	0	60
	SZW3	6/5/2009	90	100	25	15	5	50
	SZW5	05/27/2009	76	94	23	30	10	20
	SZW6	05/27/2009	88	94	29	20	0	50
	SZW8	06/23/2009	NA	NA	NA	62	56	81

^a The specific location of sites is indicated in Table 6.

^b Frequency of strains capable of inducing a hypersensitive reaction on tobacco.

^c Frequency of strains for which at least 10^{-6} cells were ice nucleation active at -8°C or warmer.

^d Frequency of strains for which at least 10^{-6} cells were ice nucleation active between -4°C and -2°C

^e Frequency of strains inducing symptoms on more than half of the cotyledons and having a mean score severity \leq

^f Frequency of inducing symptoms on more than half of the cotyledons and having a mean score severity > 2 .

^g Frequency of strains for which there was a detectable zone of inhibition (> 0 mm) of *G. candidum*.

^h Frequency of strains producing a zone of antibiosis against *G. candidum* > 2 mm.

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SYR^{>2h}

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(Auteur de correspondance) (2014). Soil water flow is a source of the plant pathogen
Pseudomonas syringae in subalpine headwaters. *Environmental Microbiology*, 16 (7), 2038-2052. DOI :
10.1111/1462-2920.12296

Strain	ζ potential (mV)			C/C ₀ avec CaCl ₂ à 0.06 g L ⁻¹	
	Mean	SD	SE	Mean	SE
CC0440	-14.96	8.77	2.26		
B728a	-10.81	5.44	1.41	0.45	0.07
CC94	-18.32	7.15	1.85	0.20	0.03
SZ-030	-24.16	5.41	1.40	0.25	0.04
Cit7	-16.08	5.70	1.47		
1448a	-21.42	7.30	1.89		
UB-370	-18.40	6.08	1.57		
USA-032	-10.69	8.67	2.24		
USA-102	-18.18	7.84	2.02	0.50	0.07
TA022	-19.50	7.65	1.97	0.43	0.06
CC1513	-21.76	5.54	1.43		
DC3000	-23.58	6.73	1.74	0.05	0.01
CC0663	-18.18	4.77	1.23		
TA020	-26.02	5.57	1.44		
TA006	-24.34	4.93	1.27		
CC1524	-26.76	5.50	1.42		
UB-246	-23.54	5.94	1.53	0.28	0.06

C/C ₀ avec CaCl ₂ à 0.7 g L ⁻¹	
Mean	SE

1.13	0.16
0.12	0.02

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Table S3 Soil core monolith characteristics and details of their treatments

Experiment Characteristics	Soil columns									
	Super Sauze			Col du Lautaret			Ceillac			
	#1	#2	#3	#1	#2	#3	#1	#2	#3	
Population sizes of indigenous bacteria in percolated water of core soil monoliths	Soil mass before rain simulation (g)	2376	2374	2274	2513	2540	2522	2356	2497	2502
	Soil mass after rain simulation (g)	2401	2403	2318	2537	2558	2541	2429	2580	2584
	Volume of water retained (mL)	25	29	44	23	18	19	73	83	83
	Mass of percolated water (g)	418	413	393	430	428	432	480	462	482
	Total volume of water injected (mL)	443	442	437	453	445	450	553	546	564
	Injection time (min)	90	90	90	90	90	90	110	110	112
	Flow (ml/mn)	4.92	4.91	4.86	5.03	4.95	5.00	5.03	4.96	5.04
	Comments	None. No active vegetation	None. No active vegetation	None. No active vegetation	Active worms. Numerous tunnels at the surface. Some vegetation.	Emission of gases testifying anoxic conditions. No active vegetation	Active worms. Numerous tunnels at the surface. Some vegetation.	None. No active vegetation	No active vegetation Mycelia at the surface	No active vegetation Mycelia at the surface
	Part of TA-022 through soil core monoliths	Soil mass after rain simulation and injection of bacterial suspension (g)	2413	2412	2331	2526	2561	2543	2460	2609
Volume of water retained (mL)		12	10	14	-11	4	2	30	29	20

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Transp strain thr m ⁱ	Mass of percolated water (g)	461	449	442	410	430	416	409	431	417
	Total volume of water injected (mL)	465	463	465	457	458	449	477	480	455

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***P*-value**
(Effect of site
according to
Kruskall Wallis
sum rank test)

- 0.051
- 0.061
- 0.027
- 0.027
- 0.027
- 0.021
- 0.055

- 0.061

- 0.027

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Tableau 4. Caractéristiques des colonnes de sol prélevées dans les Alpes du Sud.

Manipulateur	Caractéristiques	Sauze 1	Sauze 2	Sauze 3	Lautaret 1	Lautaret 2	Lautaret 3	Céillac 1	Céillac 2	Céillac 3
Lessivage des colonnes de sol	Poids avant arrosage (g)	2376.4	2373.6	2273.6	2513.3	2540.1	2522.1	2356.1	2496.8	2501.5
	Poids après arrosage (g)	2401.2	2402.6	2317.6	2536.7	2557.6	2540.7	2429.1	2580	2584
	Rétention (mL)	24.8	29	44	23.4	17.5	18.6	73	83.2	82.5
	Percolat (g)	418.1	413.1	393	429.7	427.8	431.8	479.8	462.4	481.6
	Volume d'eau appliqué (mL)	442.9	442.1	437	453.1	445.3	450.4	552.8	545.6	564.1
	Durée (min)	90	90	90	90	90	90	100	110	112
	Débit (ml/mn)	4.92	4.91	4.86	5.03	4.95	5.00	5.03	4.96	5.04
Remarques		RAS. Pas de végétation active	RAS. Pas de végétation active	RAS. Pas de végétation active	Vers de terre actifs. Turricules importants en surface. Début végétation	Forte odeur anoxie, charogne, etc... Pas de végétation	Vers de terre actifs. Turricules importants en surface. Début végétation	RAS. Pas de végétation	Pas de végétation active. Feutrage blanc un peu en surface	Pas de végétation active. Feutrage blanc un peu en surface
	Poids après apport des bactéries (g)	2413.3	2412.4	2331.2	2525.5	2561.3	2543.1	2459.5	2608.6	2603.9
	Rétention (mL)	12.1	9.8	13.6	-11.2	3.7	2.4	30.4	28.6	19.9
Transport TA022	Percolat (g)	460.6	449.2	442.1	410	430	415.9	408.8	430.7	417.2
	Volume de suspension appliqué (mL)	464.5	463.1	464.9	456.9	457.9	448.8	477.2	479.6	454.9

Table S4 Characteristics of the Fontainebleau sand

Characteristics	Fontainebleau sand
Grain diameter (μm)	230-310
Manufacturer	Merck
Column volume (cm^3)	294.5
Dry solid mass (g)	480
Density (g/cm^3)	2.65
Column density (g/cm^3)	1.61
Porosity	0.393
Pore volume (cm^3)	117.3
Pump flow (mL/min)	0.6
γ^{LW} (mJ / m^2) ^a	24.9

^a Lifshitz–van der Waals component implied in the DLVO theory