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Abundance and diversity of *Pseudomonas syringae* in surface water throughout a watershed in a Mediterranean fruit and vegetable production region

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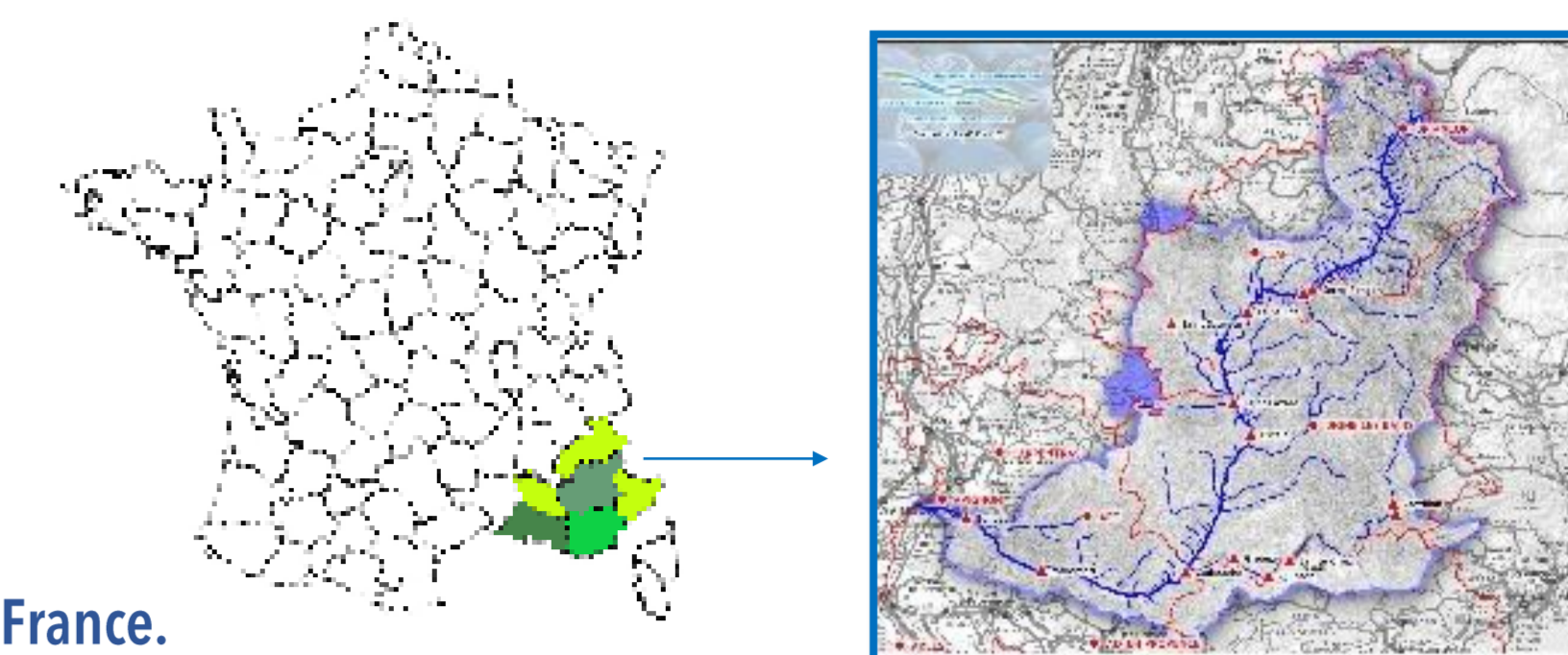
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Is *Pseudomonas syringae* present in river water along the full reach of a watershed or only in sections close to agriculture?

Other habitats of phyllosphere microorganisms

Rivers can harbor and transport plant pathogens, yet their roles in disease epidemiology and pathogen ecology have not been fully explored

River waters are widely used for irrigation including in southeastern France where 27% of France's horticultural crop-producers and 21% of its fruit orchardists are located. The Durance River - exploited since the 1100's for irrigation, milling, navigation, drinking water, mining of sediment, generation of electricity and recreation involving the creation of canals and dams, restructuration of banks and dredging of sediments - drains over 14000 km² of which 20% is agricultural production. *Pseudomonas syringae* has been isolated from the Durance and other rivers throughout the world, leading to questions about the link between irrigation and disease outbreaks [1].



Durance River basin in southeastern France.

Do *P. syringae* population sizes vary throughout a river basin?

In the SPREE project [2], we examined if populations of *P. syringae* are homogenous along the full length of the river. They could be affected by land use and seasonal changes in water flow. Ground cover and land use in the Durance River basin are influenced by topography. Recreation, pastures and nature reserves dominate the mountainous north from the origin to Lake Serre Ponçon, a reservoir of 1.2 billion m³. Crop cultivation and large urban zones dominate below the lake. Flow rates and particle content vacillate with season - mostly due to snow melt - and with climatic events.

An irrigation canal in southeastern France.



Methods

Water samples from 21 sites along a 270 km stretch of the river were collected in winter, spring, summer and fall of 2016 and 2017.

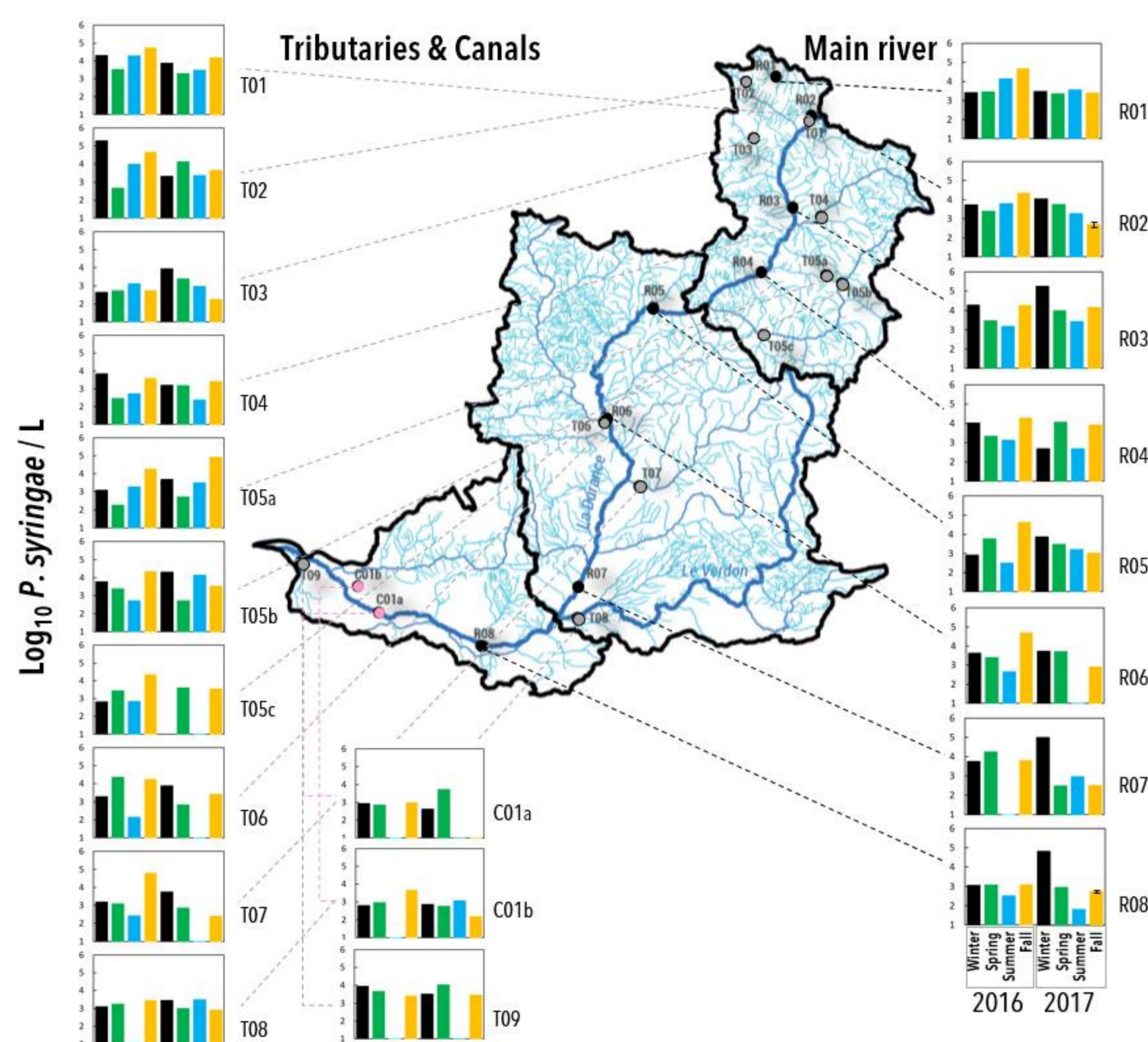
Bacteria were isolated quantitatively via dilution plating on semi-selective media. Up to 30 randomly selected *P. syringae*-like colonies were isolated for each of the 168 samples and stored in buffer in 96-well plates before analysis.

Identification of the >6000 isolates was based on the partial sequence of the citrate synthase (*cts*) gene of each isolate obtained with a modified NGS approach. They were identified with the Dada2 bioinformatics pipeline in R and with phylogenetic analyses by comparing to a database of sequences for > 900 strains of *P. syringae* representing the full known genetic diversity of this complex.

Results

1. *P. syringae* was present at all sites and in 155 of the 168 samples

Figure 1. Concentrations of *P. syringae* in river and canal water in the Durance River basin.



2. *P. syringae* population sizes were inversely correlated with temperature throughout the watershed - the strongest factor explaining variability in population size. Environmental factors had markedly different impacts on total population sizes than on *P. syringae* population sizes.

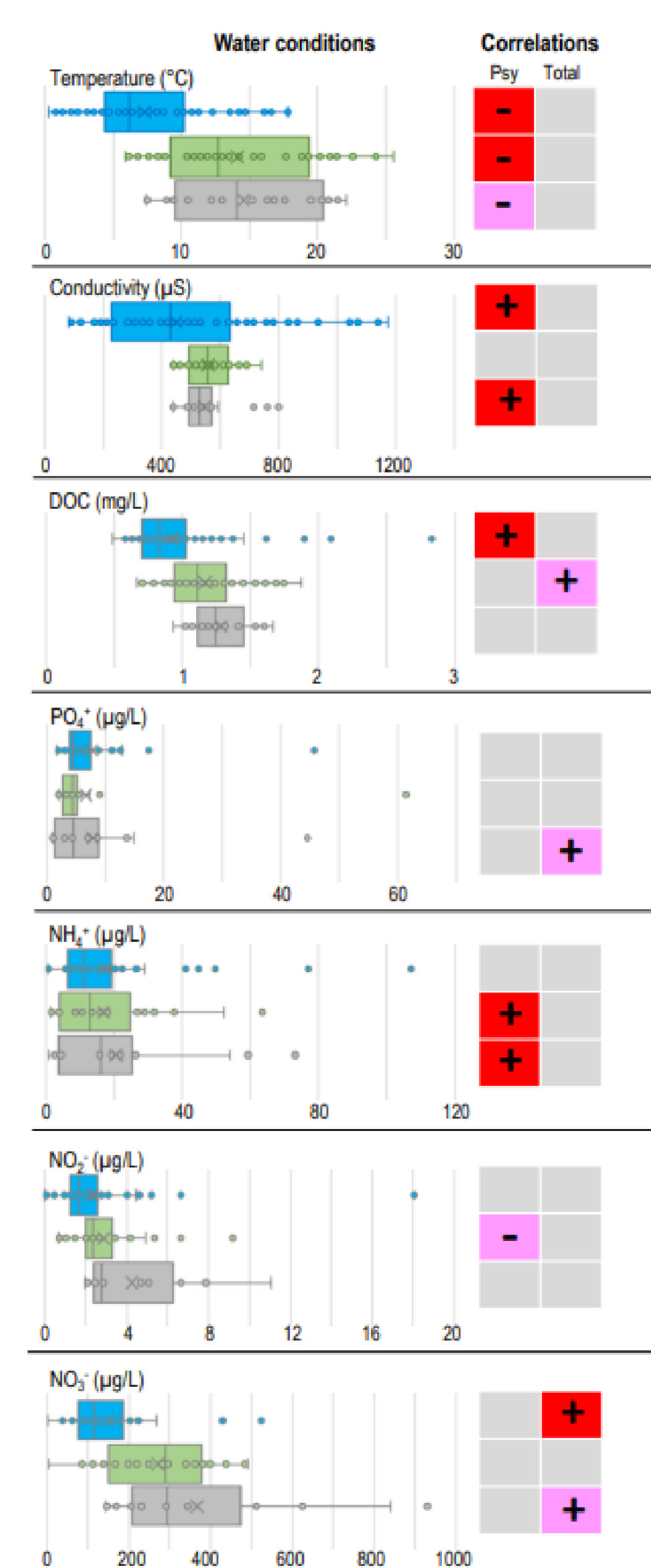
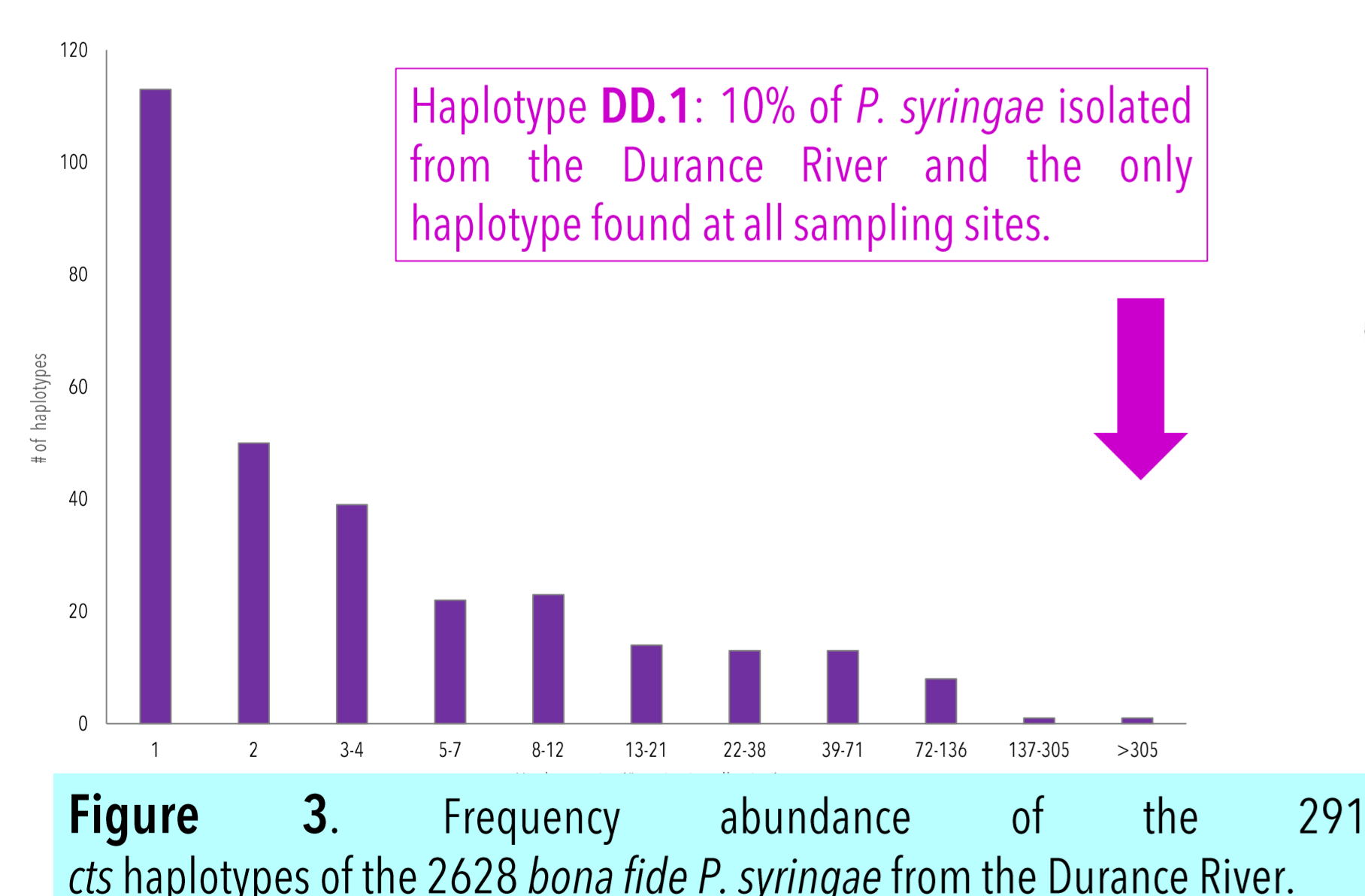


Figure 2. Correlation of *Pseudomonas syringae* (Psy) and total mesophilic (Total) bacterial population densities with water conditions in the three basins of the Durance River catchment. The left-hand panel indicates the water conditions (box plots including a presentation of all data values) in the three basins (as depicted in Fig. 1) (upper in blue, middle in green and lower in grey). The right hand panel indicates whether the values of the Spearman Rank correlation between the water conditions and each of the bacterial population densities were positive (+) or negative (-) if they were significant according to $p < 0.05$ (red background) or $0.05 > p > 0.10$ (pink background). Grey backgrounds indicate that $p > 0.10$ for this statistical test.

3. *P. syringae* populations were dominated by a *cts* haplotype that is ubiquitous worldwide and in multiple habitats



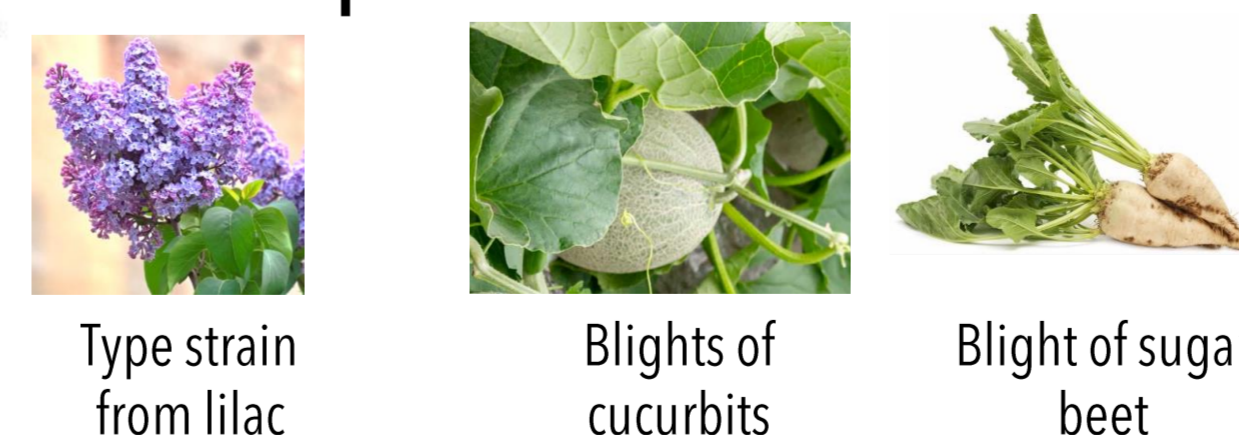
Haplotype DD.1 corresponds to reference strains isolated from >10 countries (●),



from the widest diversity of habitats,

Plants, Rain, Groundwater, Rivers, Snow fall, Leaf litter, Snowpack, Irrigation water, Epilithic biofilms

& from epidemics on several hosts.



Conclusions

P. syringae is regularly present throughout the Durance River system with temperature as a major factor contributing to the variation in population sizes across seasons and different geographic contexts. The widespread occurrence of genetic lines with epidemic potential raises questions about their origins and how to survey river water and manage its use for irrigation. In spite of the abundance of the DD.1 haplotype that has strong epidemic potential, there is relatively little disease caused by *P. syringae* in the Durance basin at present. This suggests that risk assessment could focus on indicators of deviation from the current environmental context of climate, water traits, land use and crop cultivars.

ABUNDANCE AND DIVERSITY OF *PSEUDOMONAS SYRINGAE* IN A RIVER THROUGHOUT A WATERSHED IN A MEDITERRANEAN FRUIT AND VEGETABLE PRODUCTION REGION

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[1] Morris et al. 2013. *Annu. Rev. Phytopath.*
[2] ANR project 17-CE32-0004 SPREE- Strategic preemptive pathogen surveillance of air and water to anticipate plant disease emergence in scenarios of changing land use