

## Supplementary information

Title: Biogeoclimatic regions and land-use structure bacterial biodiversity and nitrogen cycling in headwater stream sediments

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**Table S1** Description of sampling sites, including stream name, land-use type, biogeoclimatic region, geographic coordinates, and altitude

Stream	Region	Land-use	Coordinates	Altitude (m)
Vícar	Mediterranean lowlands	Agricultural	36°50'06"N 2°38'18"W	301
Las Negras	Mediterranean lowlands	Natural vegetation	36°53'30"N 2°00'36"W	69
Río Verde	Mediterranean lowlands	Forestry	36°49'58"N 3°44'16"W	552
Sierro	Mediterranean lowlands	Urban	37°19'26"N 2°23'58"W	700
Gallego	Sierra Nevada mountains	Agricultural	37°09'55"N 3°04'43"W	1243
Alhama	Sierra Nevada mountains	Natural vegetation	37°12'05"N 3°15'10"W	1373
Del Barrio	Sierra Nevada mountains	Forestry	37°09'12"N 3°08'03"W	1376
Benéjar	Sierra Nevada mountains	Urban	37°10'01"N 3°04'35"W	1236
Kolitzta	Cantabrian mountains	Agricultural	43°14'10.2"N 3°13'06.5"W	220
Cuchillo	Cantabrian mountains	Natural vegetation	43°12'45.5"N 3°16'14.7"W	305

La Tejera	Cantabrian mountains	Forestry	43°19'30.9"N 3°17'14.6"W	110
Mercadillo	Cantabrian mountains	Urban	43°16'28.5"N 3°09'12.3"W	82

**Table S2** Results of permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarities, testing the effects of biogeoclimatic region and land-use type on bacterial community composition

Factor	Df	R <sup>2</sup>	F	p-value
<b>Region</b>	2	0.221	3.471	<0.001
<b>Land-use</b>	3	0.253	2.648	<0.001

**Table S3** Functional genes involved in major nitrogen-cycling pathways, including KEGG ortholog (KO) identifiers, enzymatic functions, and associated metabolic processes

Gene	KO code	Function	Metabolic pathway
<i>amoB</i>	K10945	ammonia monooxygenase subunit B	Nitrification
<i>amoC</i>	K10946	ammonia monooxygenase subunit C	Nitrification
<i>napA</i>	K02567	nitrate reductase (cytochrome)	NO <sub>3</sub> <sup>-</sup> respiration
<i>napB</i>	K02568	nitrate reductase (cytochrome)	NO <sub>3</sub> <sup>-</sup> respiration
<i>narI</i>	K00374	nitrate reductase gamma subunit	NO <sub>3</sub> <sup>-</sup> respiration
<i>narG</i>	K00370	nitrate reductase / nitrite oxidoreductase, alpha subunit	NO <sub>3</sub> <sup>-</sup> respiration
<i>narH</i>	K00371	nitrate reductase / nitrite oxidoreductase, beta subunit	NO <sub>3</sub> <sup>-</sup> respiration
<i>nirB</i>	K00362	nitrite reductase (NADH) large subunit	NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup> assimilation
<i>nasC</i>	K00372	assimilatory nitrate reductase catalytic subunit	NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup> assimilation
<i>nrtC</i>	K15578	nitrate/nitrite transport system ATP-binding protein	NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup> assimilation
<i>nirA</i>	K00366	ferredoxin-nitrite reductase	NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup> assimilation
<i>nirD</i>	K00363	nitrite reductase (NADH) small subunit	NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup> assimilation
<i>narB</i>	K00367	ferredoxin-nitrate reductase	NO <sub>3</sub> <sup>-</sup> -NO <sub>2</sub> <sup>-</sup> assimilation
<i>nirS</i>	K15864	nitrite reductase	Denitrification
<i>norB</i>	K04561	nitric oxide reductase subunit B	Denitrification

<i>norC</i>	K02305	nitric oxide reductase subunit C	Denitrification
<i>nosZ</i>	K00376	nitrous-oxide reductase	Denitrification
<i>nirK</i>	K00368	nitrite reductase	Denitrification
<i>nifD</i>	K02586	nitrogenase molybdenum-iron protein alpha chain	N fixation
<i>nifK</i>	K02591	nitrogenase molybdenum-iron protein beta chain	N fixation
<i>anfG</i>	K00531	nitrogenase delta subunit	N fixation
<i>nifH</i>	K02588	nitrogenase iron protein	N fixation
<i>gltD</i>	K00266	glutamate synthase (NADPH) small chain	NH <sub>4</sub> <sup>+</sup> assimilation
<i>GLUD1_2</i>	K00261	glutamate dehydrogenase (NAD(P)+)	NH <sub>4</sub> <sup>+</sup> assimilation
<i>glnA</i>	K01915	glutamine synthetase	NH <sub>4</sub> <sup>+</sup> assimilation
<i>nrfH</i>	K15876	cytochrome c nitrite reductase small subunit	DNRA
<i>nrfA</i>	K03385	nitrite reductase (cytochrome c-552)	DNRA
<i>hcp</i>	K05601	hydroxylamine reductase	Unassigned