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Evaluation of the ISO standard 11063 "Soil DNA Extraction Procedure" for Assessing Microbial Abundance and Community Structure

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Introduction

For the last two decades, soil microbial diversity studies have been dependent upon the extraction and characterization of soil DNA. Therefore, the DNA extraction procedure has become a critical step in describing soil microbial biodiversity. Numerous soil DNA extraction procedures are available, including a standardized one (ISO 11063). All these procedures rely on physical and chemical lysis steps, and are adapted to molecular tools like qPCR or fingerprinting techniques. The recent development of massively parallel sequencing technologies which permit more representative and accurate taxonomical inventories, has instigated a reassessment of the biases associated with the DNA extraction procedure. This study aims to give an alternate procedure for extracting soil microbial DNA, which could give a less biased picture of soil microbial diversity.

Experimental procedures

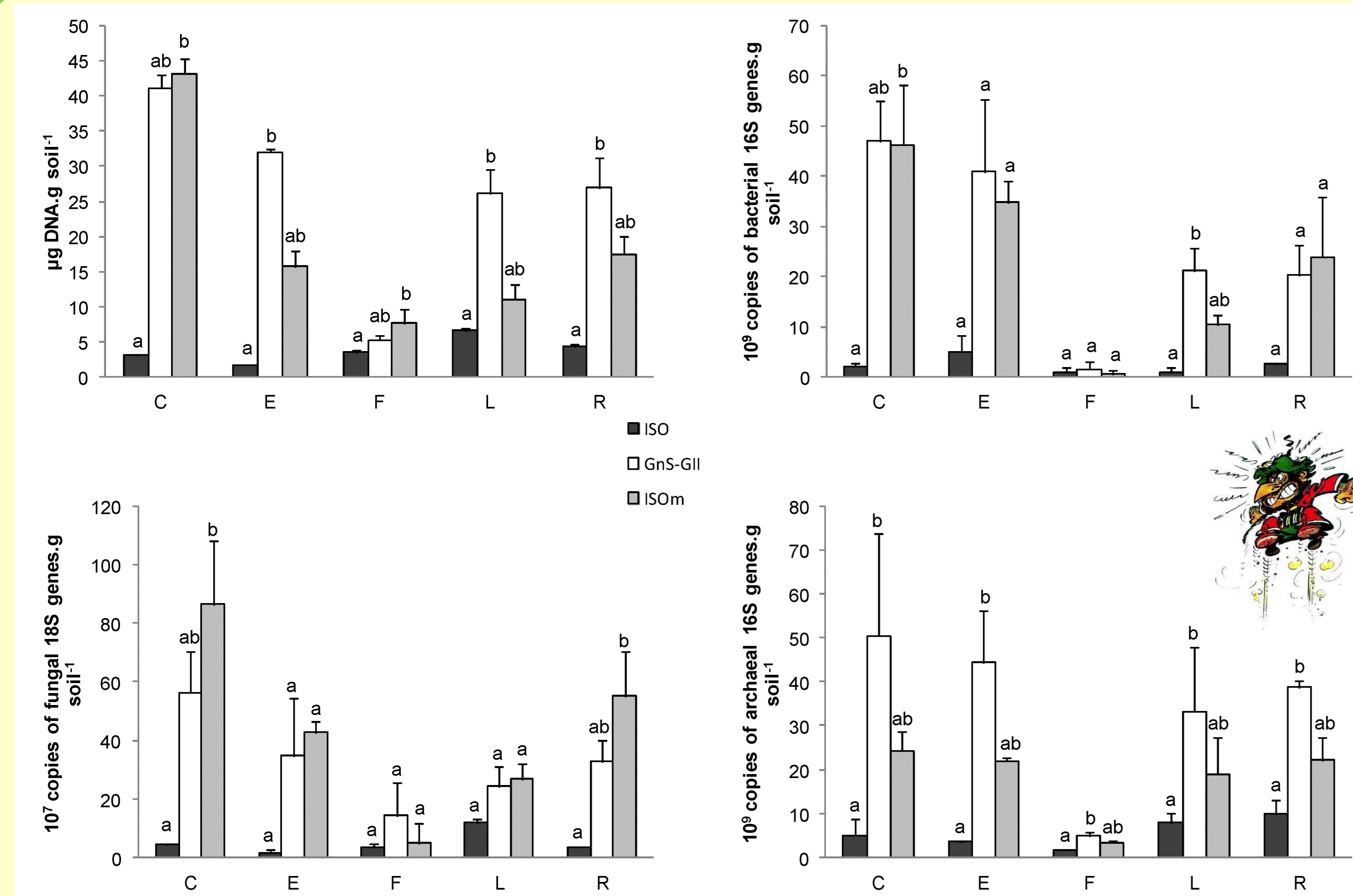
		ISO	GnS-GII	ISOm
beads	soil	1g	1g	1g
	glass beads 106 µm	2g	-	-
	glass beads 2 mm	8	-	-
	ceramic beads 1.4 mm	-	2.5g	2.5g
	glass beads 8 mm	-	4	4
lysis buffer	silica beads 100 µm	-	2g	2g
	lysis buffer 1 *	4ml	-	4ml
	lysis buffer 2 *	-	4ml	-
grinding	bead biting	1600 rpm, 30 sec	-	-
	fastprep	-	3*30sec, 4m/sec	3*30sec, 4m/sec
chemical lysis	70°C	10 min	2*15min	2*15min
	supernatant recovery	Centrifugation	1 min, 14000g	5 min, 7000g
Deproteinisation (for 1 ml supernatant)	Potassium acetate pH5.5	100µl	100µl	100µl
	incubation on ice	10 min	10 min	10 min
	Centrifugation	5 min, 14000g	5 min, 14000g	5 min, 14000g
DNA precipitation	isopropanol -20°C	900µl	900µl	900µl
	incubation -20°C	15 min	30min	30min
	centrifugation	30min, 14000g	30 min, 13000rpm	30 min, 13000rpm
	ethanol 70% -20°C	400µl	400µl	400µl
	centrifugation	15 min, 14000g	5 min, 13000rpm	5 min, 13000rpm
	DNA pellets drying	15 min, 37°C	15 to 25 min, 50°C	15 to 25 min, 50°C
DNA suspension (U.P. water)	100µl	100µl	100µl	

Soil	Collection site	Origin	Clay (mg.g ⁻¹)	Fine loam (mg.g ⁻¹)	Coarse loam (mg.g ⁻¹)	Fine sand (mg.g ⁻¹)	Coarse sand (mg.g ⁻¹)	Organic Carbon (mg.g ⁻¹)	Total N (mg.g ⁻¹)	C/N	CaCO ₃ (mg.g ⁻¹)	pH
C	Agricultural Site	Crop soil	504	180	145	73	98	24.9	2.8	9	102	7.75
E	INRA Experimental Site	Crop soil	392	320	228	34	26	16.5	1.65	10	2	7
F	Forest Observatory Plot	Forest soil	101	167	205	217	310	103.3	3.1	34	<1	3.8
L	INRA Experimental Site ACBB Lusignan	Grassland	175	369	304	73	79	13.2	1.33	9.92	<1	6.6
R	Agricultural Experimental Site	Crop soil	79	66	44	315	496	50.2	2.16	23.3	22	7.5

3 DNA extraction protocols tested on 5 contrasted soils

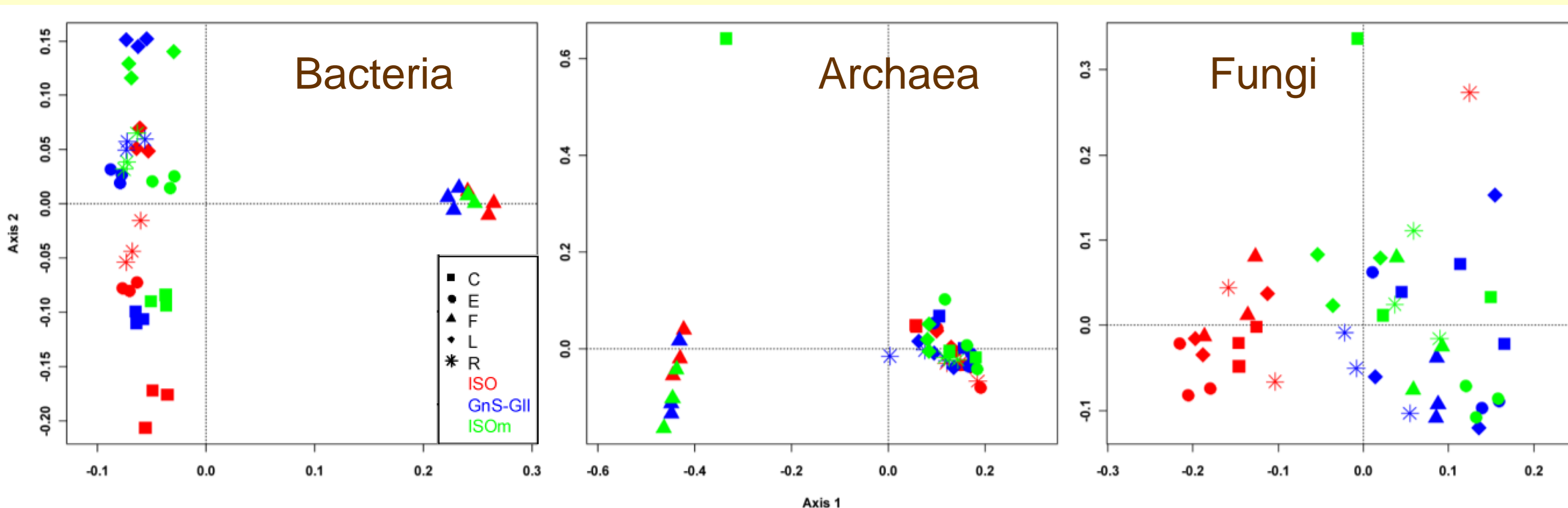
Extracted DNA quality evaluation:

- Total extracted DNA quantification
- 16S and 18S gene quantification (qPCR)
- Diversity patterns (tRFLP) for bacteria, archaea, fungi



Results

- ➔ GnS-GII and ISOm protocols yield as much or more DNA than the ISO procedure
- ➔ ISOm yields an average of 1.9 times more fungal DNA than the standardized method



	Bacteria		Archaea		Fungi	
	F	R ²	F	R ²	F	R ²
ISO	56.39	0.96*	50.62	0.95*	2.09	0.46*
GnS-GII	37.38	0.94*	34.29	0.93*	8.41	0.77*
ISOm	31.34	0.93*	9.48	0.79*	9.29	0.79*

PerMANOVA analysis showing the effect of soil type on microbial communities assessed by the three extraction methods. *denotes significance ($p < 0.01$).

- ➔ GnS-GII and ISOm better discriminate soil type differences in fungal communities

- ➔ The three procedures allow distinction of communities coming from different soil types
- ➔ Soil grinding procedure (Bead Beating / FastPrep) has a strong impact on the observed fungal diversity

Conclusions - Perspectives

- ➔ A simple modification of the ISO procedure (i.e. FastPrep grinding) leads to a higher microbial DNA extraction yield and to a better discrimination of soil microbial communities.
- ➔ The ISO is good for studying bacteria, but the ISOm is better to study microbial communities.
- ➔ 454 pyrosequencing will confirm which of these procedures is better adapted to study complex communities