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1 **Short communication**

2

3 **Assessing the effect of organic residue quality on active decomposing fungi in a tropical**

4 **Vertisol using ^{15}N-DNA stable isotope probing**

5

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18 **Running title:** Residue quality controls active decomposing soil fungi.

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Abstract (150 words)

^{15}N-DNA stable isotope probing (^{15}N-DNA-SIP) combined with 18S rRNA gene-based community analysis was used to identify active fungi involved in decomposition of ^{15}N-labeled maize and soybean litter in a tropical Vertisol. Phylogenetic analysis of ^{15}N-labeled DNA subjected to 18S rRNA gene-based community fingerprinting showed that organic residue quality promoted either slow (i.e. *Penicillium* sp., *Aspergillus* sp.) or fast growing (i.e. *Fusarium* sp., *Mortierella* sp.) fungal decomposers in soils treated with maize or soybean residues, respectively, whereas *Chaetomium* sp. were found as dominant decomposers in both residue treatments. Therefore, we have clear evidence that specific members of the fungal community used ^{15}N derived from the two different organic resources for growth and stimulated early decomposition of maize or soybean decomposition. In conclusion, our study showed that ^{15}N-DNA-SIP-based community analyses cannot only follow the flow of N from organic resources into bacteria, but also into the actively decomposing fungal communities of soils.

Keywords

^{15}N-DNA stable isotope probing, active decomposing fungi, tropical soil, plant residue quality.

45 **Entire text** (1486 words)

46 Soil fungi represent a major proportion of soil microbial biomass and have been
47 acknowledged to be predominant decomposers of organic matter in tropical soils (Rillig *et al.*
48 2001; Yang & Insam 1991; Lodge 1985). However, the relationship between fungal
49 community dynamics and fungal decomposition processes in tropical soils remains is
50 relatively unexplored and thus needs further investigation to improve our current
51 understanding of the specific contributions of fungi to organic residue decomposition and
52 nutrient cycling in soils (Gomes *et al.* 2003). Although there has been progress in
53 understanding the nature of bacterial communities contributing to crop residue decomposition
54 (Bernard *et al.* 2007), knowledge of fungal communities is limited, and generally restricted to
55 cultivation-dependent studies (e.g. Robinson *et al.* 1994).

56 The development of nucleic acid-based, cultivation-independent approaches has opened new
57 avenues for the sensitive detection of microbial communities in terrestrial ecosystems (Theron
58 & Cloete, 2000), but these have rarely been used to study fungal communities in tropical soil
59 ecosystems (Gomes *et al.* 2003). Since the advent of combining molecular techniques with
60 stable isotopes (e.g. ^{13}C, and ^{15}N), DNA-based stable isotope probing (DNA-SIP, Radajewski
61 *et al.* 2000) has became a powerful tool to achieve better understanding of microbial
62 processes through identifying relevant organisms that determine nutrient cycling and
63 metabolize plant-derived carbonaceous compounds (Rasche *et al*, 2009; Bernard *et al*, 2007;
64 el Zahar Haichar *et al.* 2007). While ^{13}C-based DNA-SIP has focused on the C cycle, ^{15}N-SIP
65 is particularly attractive to trace microbial processes involved in N cycling (Buckley *et al.*
66 2008; Buckley *et al.* 2007; Cupples *et al.* 2007). However, ^{15}N-DNA-SIP has to our
67 knowledge not been used to investigate the role of soil fungi involved in the decomposition of
68 complex plant residues.

69 In a previous study, we have proven that ^{15}N-DNA-SIP combined with 16S rRNA gene-based
70 community fingerprinting was appropriate to detect active bacteria involved in the
71 decomposition of complex, ^{15}N-labeled plant residues of different quality (i.e. maize and
72 soybean) in a tropical Vertisol (España *et al*. in revision). Based on this preliminary study, we
73 hypothesized that ^{15}N-DNA-SIP along with 18S rRNA gene-based community fingerprinting
74 was suited to assess the effect of organic residue quality on active members of the
75 decomposing fungal community in the previously assayed tropical soil.

76 ^{15}N-enriched leaf residues (90 atom-%), i.e. maize (*Zea mays* L.) (total nitrogen (N_t) content
77 1.21%; C-to-N_t ratio: 32; cellulose content: 24.9%) and soybean (*Glycine max* L. Merr.)
78 (2.71%; 15; 15.5%), and an unlabeled control of both residues were incubated (1%) under
79 controlled conditions (40% water holding capacity, 25°C) in topsoil (0-20 cm) of a Vertisol
80 taken from a long-term field experiment in Venezuela (Rodriguez *et al.*, 2004). After 15 days
81 incubation, DNA was extracted from 0.3 g fresh soil (FastDNA Spin Kit for soil, MP
82 Biomedicals, Solon, USA), and quantified (BioPhotometer 6131, Eppendorf, Hamburg,
83 Germany). Isopycnic fractionation (SIP) of labeled and unlabeled DNA was performed
84 according to Cadisch *et al*. (2005) and Hutchens *et al*. (2004). DNA density gradients were
85 purified (Sambrook *et al*., 1989), and DNA from density-resolved SIP fractions was
86 quantified as described above. ^{15}N-enrichment of DNA fractions was determined according to
87 España et al. (in revision). Fungal 18S rRNA genes were amplified by a semi-nested PCR
88 protocol using a ready-to-use PCR mix (Biomix, Bioline, Luckenwalde, Germany). The first
89 PCR (PCR 1) was performed in 25 µl reactions containing 12.5 µl ready-to-use PCR mix, 30
90 ng DNA of each fraction, oligonucleotides (0.2 mM each) NS1 and EF3 (Oros-Sichler *et al*.
91 2006; Fisher Scientific, Schwerte, Germany) and ultra-pure PCR water (Roth, Karlsruhe,
92 Germany). PCR 1 was performed with 94°C for 5 min; 25 cycles at 94°C for 30 s, 47°C for
93 45 s and 72°C for 3 min; and a final extension at 72°C for 10 min. Two µl of PCR 1 were

94 used as template for the second amplification (PCR 2) with oligonucleotides NS1 (Oros-
95 Sichler *et al*. 2006) and NS2-GC (Marschner *et al*. 2002) (0.2 mM each, Fisher Scientific).
96 PCR 2 was run with 94°C for 5 min, 80°C for 10 min, 35 cycles at 94°C for 30 s, 47°C for 45
97 s and 72°C for 3 min, and a final extension at 72°C for 10 min. Amplicons were checked in
98 2% Sybr® Green stained agarose gels (Sigma-Aldrich, Munich, Germany) and subjected to
99 denaturing gradient gel electrophoresis (DGGE) analysis according to España et al. (in
100 revision). Three independent DGGE analyses per fraction were performed for each sample to
101 verify the method reproducibility. Four bands revealing distinct changes of their relative
102 intensity along the gradient (from 'light' unlabeled to 'heavy' ^{15}N-labeled DNA fractions)
103 were selected for cloning and sequencing analysis (Fig. 1; bands: maize (M): M1 to M4;
104 soybean (S): S1 to S4). DGGE gel bands were purified and used for generating the 18S rRNA
105 gene libraries according to España et al. (in revision). From each library, four positive clones
106 were partially sequenced (GATC Biotech, Konstanz, Germany) with reverse M13 primer and
107 sequence information (approximately 550 bases) was subjected to BLAST analysis with the
108 National Center for Biotechnology Information (NCBI) database. Sequences were deposited
109 in Genbank under accession numbers HM475173 to HM475268.
110 Our study showed that ^{15}N-DNA-SIP was suited to asses active decomposing fungi in both
111 ^{15}N-labeled plant residues evaluated and proved that ^{15}N-DNA-SIP was useful for fungal
112 decomposition studies using organic materials with contrasting biochemical composition. A
113 prerequisite to the successful application of ^{15}N-DNA-SIP is the use of highly ^{15}N-enriched
114 (at least 90 atom%) residues allowing a sufficient labeling of soil DNA to obtain reliable, ^{15}N-
115 enriched SIP fractions (Fig. 1) (Cadisch *et al.* 2005) and to identify the active decomposing
116 community, either fungi (this study) or bacteria, as was shown by España *et al*. (in revision).
117 However, the use of unlabeled residues as control is mandatory to eliminate the effect of
118 different G+C contents (Buckley *et al.* 2007; Cupples *et al.* 2007; Neufeld *et al.* 2007).

119 Residue incorporation induced remarkable differences in the community structure by inducing
120 new bands in the 18S rRNA gene DGGE gels (patterns labeled with "M" for maize, and "S"
121 for soybean), which were not present in the control (C) treatment (without residue) (Fig. 1). In
122 both residue treatments, unlabeled control treatments revealed only small community changes
123 compared to those of the ^{15}N-labeled treatments. Several bands increased (e.g. M1, M2, M4,
124 S1, S2, and S4) in relative intensity from 'light' (fraction #5, 2.4 atom-% (soybean), and 0.06
125 atom-% (maize), Fig. 1) to 'heavy' (fraction #1, 87.1 atom-% (soybean), and 57.8 atom-%
126 (maize), Fig. 1) DNA fractions, or remained constant (M3, and S3).
127 Phylogenetic assignment of cloned bands revealed that the majority of sequences obtained
128 from both maize and soybean treatments were affiliated with *Chaetomium* sp. (bands M3, M4,
129 S3, and S4), while sequences obtained from bands S1 and S2 were related to *Fusarium* sp.
130 and *Mortierella* sp., respectively. These active species belong to *Ascomycota*, known as key
131 players in organic residue decomposition (Thorn & Lynch 2007; Kjøller & Struwe 2002;
132 Montgomery *et al*. 2000). *Chaetomium* sp. and *Fusarium* sp. are known to degrade a range of
133 contrasting and complex organic materials (e.g. maize and soybean residues) (Shaheen *et al*.
134 2008; Katapodis *et al*. 2007), while *Mortierella* sp. has been shown to utilize mainly easy
135 degradable C compounds (Thorn & Lynch 2007). Interestingly, *Penicillium* sp. and
136 *Aspergillus* sp., slow growing fungi, and important in the initial cellulose degradation
137 (Horwath 2007), were identified only in the maize treatment (bands M1 and M2) evincing
138 almost twice as much cellulose than soybean residue. Contrastingly, *Fusarium* sp. and
139 *Mortierella* sp., which were only found in the soybean treatment, are considered to be fast
140 growing fungi (Thorn & Lynch 2007; Kjøller & Struwe 2002; Montgomery *et al*. 2000). We
141 assumed that the fast fungal response to soybean residue addition was due to the development
142 of opportunistic fungi feeding on relatively easily degradable plant residue constituents such
143 as glucose and proteins rather than cellulous components. Although it has been shown that

144 readily decomposable compounds are mainly utilized by bacteria, fast growing opportunistic
145 fungi have recently been found to also be stimulated by easy accessible C sources and high N
146 availability (Poll *et al.* 2010; Rousk & Bååth 2007; van der Wal *et al*. 2006).
147 In conclusion, ^{15}N-DNA-SIP along with 18S rRNA gene-based community profiling was a
148 powerful approach to follow the flow of N from contrasting organic resources into the fungal
149 communities actively decomposing complex maize and soybean residues in a tropical
150 Vertisol. Indications were provided that certain fungi were essentially involved in early stages
151 of organic matter decomposition. However, we investigated only some prominent DGGE
152 bands in more detail and therefore recommend for future research to extend community
153 profiling approaches with studying total fungal communities to assess their richness and
154 abundance as well as relevant functional genes to better understand the essential contribution
155 of soil fungi in specific decomposition processes and nutrient cycling.
156
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160
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238

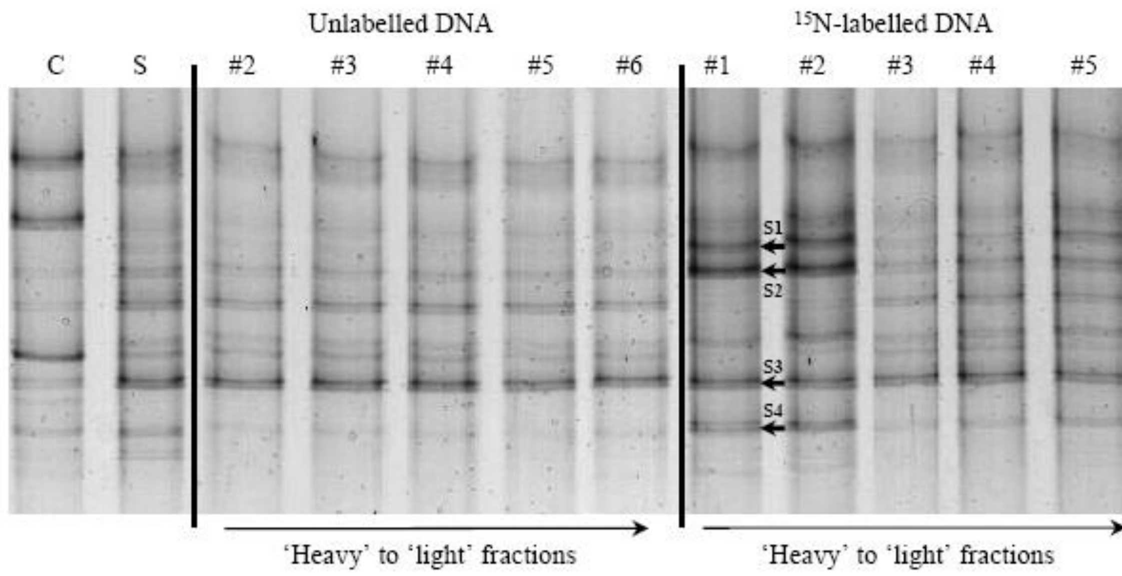
239 **Figure caption**

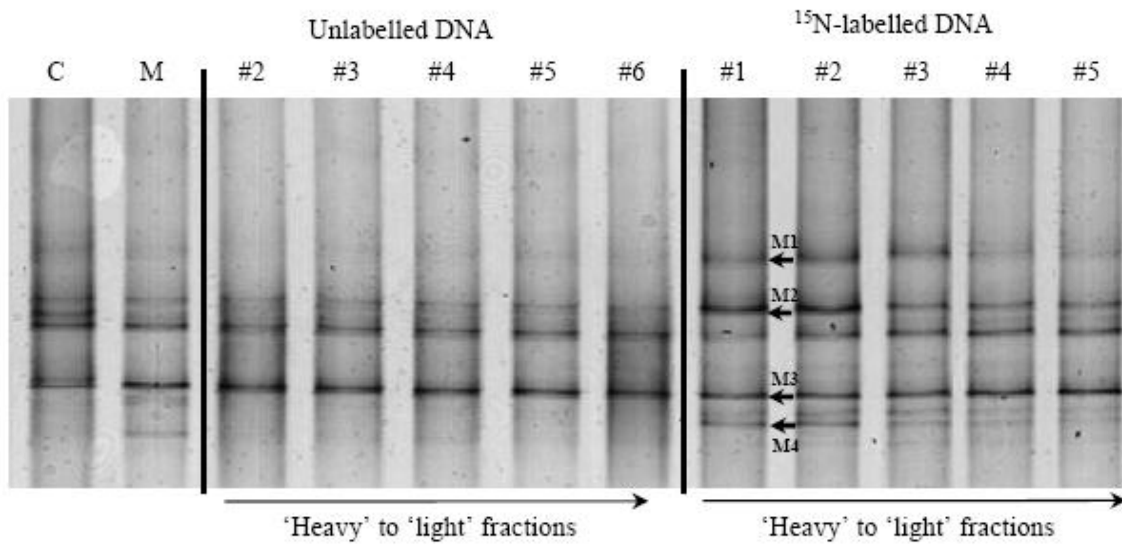
240

241 **Figure 1**

242 Denaturing gradient gel electrophoresis (DGGE) patterns of 18S rRNA genes obtained from
243 DNA fractions along the caesium chloride (CsCl) density gradient (fraction #1 ('heavy') to
244 fraction #6 ('light') from unlabeled control and ^{15}N-labeled soybean (A) and maize (B)
245 residue treatments. ^{15}N-enrichment in labeled fractions: soybean (fraction #1 (87.1 atom-%),
246 #2 (44.6 atom-%), #3 (20.4 atom-%), #4 (3.02 atom-%), and #5 (2.4 atom-%)), and maize
247 (fraction #1 (57.8 atom-%), #2 (22.0 atom-%), #3 (19.2 atom-%), #4 (0.24 atom-%), and #5
248 (0.06 atom-%)). DGGE pattern coding: C = DGGE pattern of control without any residue
249 treatment; M/S = DGGE pattern of non-CsCl-fractionated soil DNA from soybean (S) and
250 maize (M) residue treatment. Arrows indicate the DGGE-bands which have been selected for
251 cloning and sequencing analysis. Affiliation of sequenced clones (12 clones per band):
252 *Fusarium* sp. (Ascomycetes, band S1; 9 clones with associated Genbank accession number
253 (closest NCBI match): EJ613599, homology: 98-100%; 2 clones: AB110910, 99-100%; 1
254 clone: GQ166777, 98%), *Mortierella* sp. (Zygomycetes; band S2; 4 clones: AY129549, 99%;
255 3 clones: EU736291, 98%; 2 clones: AY550125, 100%; 2 clones: AF113425, 99-100%; 1
256 clone: AY546098, 99%), *Penicillium* sp. (Ascomycetes; band M1; 4 clones: AF245241, 98-
257 99%; 4 clones: AF245268; 99-100%; 2 clones: AF245267, 98-99%; 1 clone: GU190185,
258 99%; 1 clone: AF245245, 99%), *Aspergillus* sp. (Ascomycetes; band M2; 5 clones:
259 EF033516, 98-99%; 2 clones: AP007173, 98-99%; 2 clones: FJ393420, 99-100%; 2 clones:
260 EU884135, 98-100%; 1 clone: AB048285, 100%), and *Chaetomium* sp. (Ascomycetes; bands
261 S3, S4, M3, M4; all clones: FJ393436, 98-100%). Note: from fractions #1 of the unlabeled
262 control treatments and fractions #6 of the ^{15}N-labeled treatments, no 18S rRNA gene
263 amplicon could be achieved.

264





265

266 **Figure 1**