

Supplementary materials

Microbial diversity and ecosystem functioning in deadwood of black pine of a temperate forest

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Table S1. Description of visual characteristics used to assign the decay class.

Decay class	Description
1	Recently dead - small branches, wood texture intact, visible little rotten areas under bark
2	Weakly decayed - twigs partly present, wood texture intact, rotten areas < 3cm
3	Medium decay - no twigs, wood texture partly broken, rotten area > 3cm
4	Very decay - no twigs, wood soft, texture with blocky pieces, large rotten areas
5	Almost decomposed - no twigs, wood soft, powdery texture, very large rotten areas with musk and lichens

Source: modified by Paletto and Tosi (2009).

Table S2. Primer pairs used for real time PCR absolute quantification of the different microbial groups assessed in the five deadwood decay classes.

Target group	Source for standard: plasmid containing a sequence of	Primers
Bacteria	16S rDNA from <i>Pseudomonas nitroreducens</i> DSMZ1650	338F-518R (Sánchez <i>et al.</i> , 2007)
Actinobacteria	16S rDNA from <i>Streptomyces</i> sp. A2	243F-513R (Heuer <i>et al.</i> , 1997)
Fungi	18S rDNA from uncultured fungus from soil	EF390-FR1 (Vainio and Hantula, 2000)
Methanogenic Archaea	16S rDNA from uncultured archaeon from rice soil	Unimet1F-Unimet1R (Zhou <i>et al.</i> , 2009)
Methanotrophs type I	16S rDNA from <i>Methylococcus capsulatus</i> ATCC33009	MB10γ- 533R (Henckel <i>et al.</i> , 1999)
Methanotrophs type II	16S rDNA from <i>Methylocystis rosea</i> DSMZ17261	MB9α- 533R (Henckel <i>et al.</i> , 1999)
Diazotrophs	<i>nifH</i> gene from <i>Sinorhizobium meliloti</i> strain A332	polF-polR (Poly <i>et al.</i> , 2001)
Nitrifying bacteria	<i>amoA</i> gene from <i>Nitrosomonas europaea</i> NCIMB11850	AmoA1F-AmoA2R (Xu <i>et al.</i> , 2012)
Nitrifying archaea	<i>amoA</i> gene from uncultured archaeon from soil	archamoAF-archamoAR (Xu <i>et al.</i> , 2012)
Denitrifying	<i>nirK</i> gene from <i>Ochrobactrum anthropi</i> DSMZ6882	F1ACu-R3Cu (Throback <i>et al.</i> , 2004)
Denitrifying	<i>nosZ</i> gene from <i>Pseudomonas stutzeri</i> DSMZ6082	nosZF-nosZ1733R (Throback <i>et al.</i> , 2004)

Table S3. Maximum values of CO₂, CH₄ and N₂O production from black pine deadwood cores under laboratory experiment. Mean values according to each decay classes are also reported (standard errors in parentheses). Different letters in a column indicate significant differences at P<0.05 (LSD test) among means.

Decay class	Core	CO ₂ max (mg CO ₂ g ⁻¹)	CH ₄ max (mg CH ₄ g ⁻¹)	N ₂ O max (mg N ₂ O g ⁻¹)
1	1	1.49	5.2 10 ⁻⁷	-4.3 10 ⁻⁶
	2	0.38	5.4 10 ⁻⁷	-4.4 10 ⁻⁶
	3	1.11	4.4 10 ⁻⁶	-6.8 10 ⁻⁶
	4	0.63	9.7 10 ⁻⁶	-4.1 10 ⁻⁶
	mean (st error)	0.90 (0.25) bc	3.8 10⁻⁶ (2.2 10⁻⁶)	-4.9 10⁻⁶ (6.4 10⁻⁷)
2	5	0.22	-2.6 10 ⁻⁶	-7.7 10 ⁻⁶
	6	0.81	2.7 10 ⁻⁷	-1.6 10 ⁻⁵
	7	0.66	1.7 10 ⁻⁵	-7.3 10 ⁻⁶
	8	0.31	7.0 10 ⁻⁷	-4.6 10 ⁻⁶
	mean (st error)	0.50 (0.14) c	3.9 10⁻⁶ (4.5 10⁻⁶)	-8.8 10⁻⁶ (2.4 10⁻⁶)
3	9	2.07	-3.8 10 ⁻⁸	1.5 10 ⁻⁴
	10	1.12	4.4 10 ⁻⁶	-1.3 10 ⁻⁵
	11	1.04	5.4 10 ⁻⁶	-6.3 10 ⁻⁶
	12	0.93	6.3 10 ⁻⁴	-8.0 10 ⁻⁶
	mean (st error)	1.29 (0.26) ab	1.6 10⁻⁴ (1.6 10⁻⁴)	3.1 10⁻⁵ (4.0 10⁻⁵)
4	13	1.42	-2.1 10 ⁻⁷	-9.3 10 ⁻⁶
	14	1.25	9.9 10 ⁻⁷	-1.3 10 ⁻⁵
	15	1.88	5.1 10 ⁻⁶	-3.2 10 ⁻⁶
	16	1.53	-2.1 10 ⁻⁶	-9.3 10 ⁻⁶
	mean (st error)	1.52 (0.13) ab	9.3 10⁻⁷ (1.5 10⁻⁶)	-8.8 10⁻⁶ (2.1 10⁻⁶)
5	17	0.99	6.6 10 ⁻⁷	-4.4 10 ⁻⁶
	18	1.54	-8.2 10 ⁻⁷	-1.7 10 ⁻⁵
	19	2.83	-2.4 10 ⁻⁶	-1.7 10 ⁻⁵
	20	1.68	-7.3 10 ⁻⁶	-4.2 10 ⁻⁵
	mean (st error)	1.76 (0.39) a	-2.5 10⁻⁶ (1.7 10⁻⁶)	-2.0 10⁻⁵ (7.8 10⁻⁶)

Table S4. ANOSIM and PERMANOVA global test based on the Bray-Curtis similarity matrices of 18S- and 16S-rDNA DGGE, for microbial groups (fungi, bacteria, and actinobacteria) decaying downy birch deadwood.

	ANOSIM		PERMANOVA	
	R	P	F	P
Fungi	0.66	0.0001	2.77	0.0001
Bacteria	0.63	0.0001	3.87	0.0001
Actinobacteria	0.54	0.0001	3.75	0.0001

Table S5. Values of ANOSIM statistic R (upper right side) and PERMANOVA statistic F (lower left side) from pairwise comparison of the banding profiles of fungal 18S-rDNA DGGE, and bacterial and actinobacterial 16S-rDNA DGGEs.

<i>Fungi</i>	Class 1	Class 2	Class 3	Class 4	Class 5
Class 1		0.68*	0.77*	0.77*	0.99*
Class 2	2.77*		0.58*	0.57*	0.91*
Class 3	2.72*	2.00*		0.25	0.51*
Class 4	3.19*	2.26*	1.57		0.56
Class 5	5.75*	3.71*	2.15*	2.78	
<i>Bacteria</i>					
Class 1		0.72*	0.73*	0.82*	0.70*
Class 2	2.91*		0.92*	0.85*	0.89*
Class 3	4.69*	5.42*		0.76*	0.56*
Class 4	5.44*	5.37*	3.71*		0.32*
Class 5	3.21*	3.80*	2.53*	1.75*	
<i>Actinobacteria</i>					
Class 1		0.64*	0.74*	0.90*	0.85*
Class 2	2.49*		-0.06	0.42*	0.65*
Class 3	3.051*	0.67		0.89*	0.91*
Class 4	5.85*	2.92*	6.37*		0.44
Class 5	5.15*	3.77*	5.85*	3.12*	

*Significant value ($P < 0.05$)

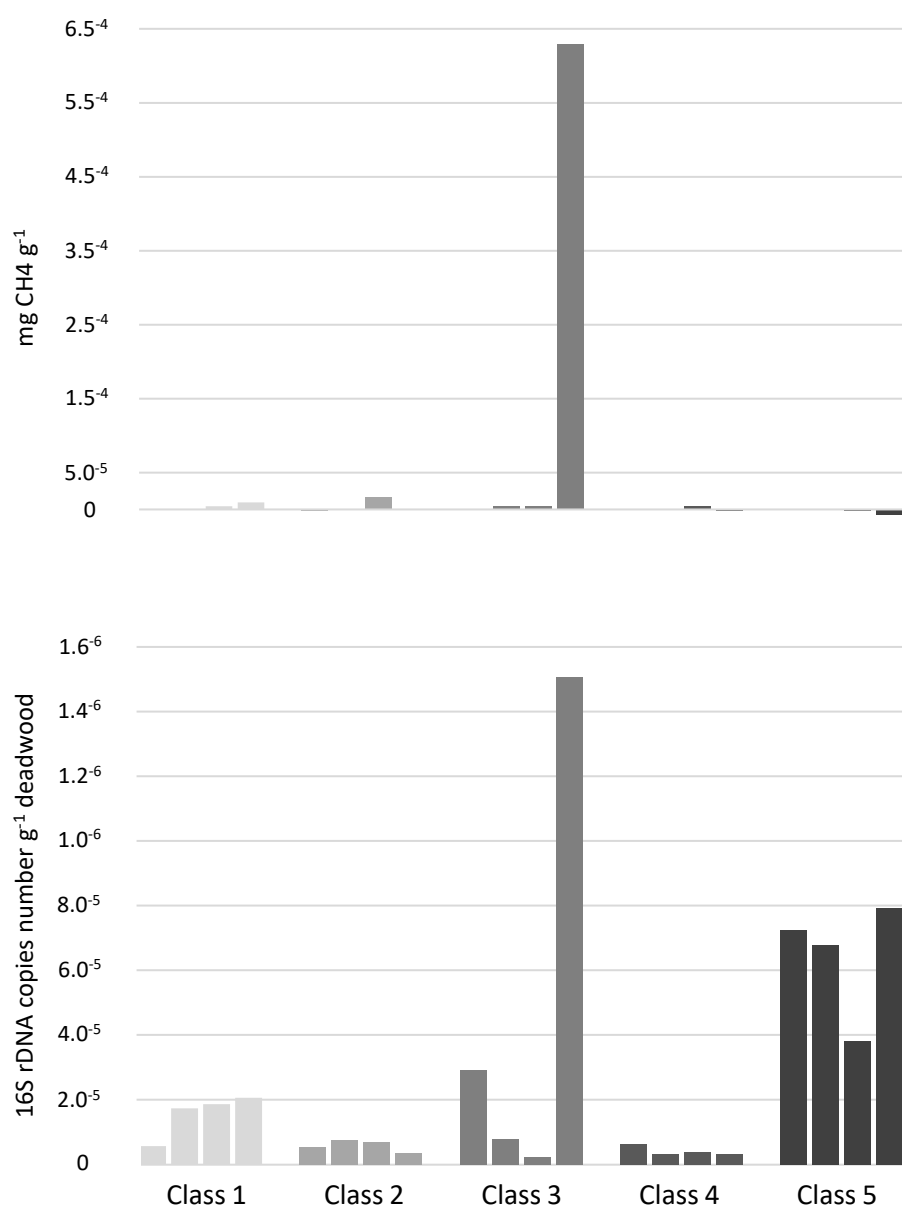


Figure S1. Maximum values of CH₄ production (top) and archaeal methanogen 16S rDNA gene copies number (bottom) from each black pine deadwood sample.

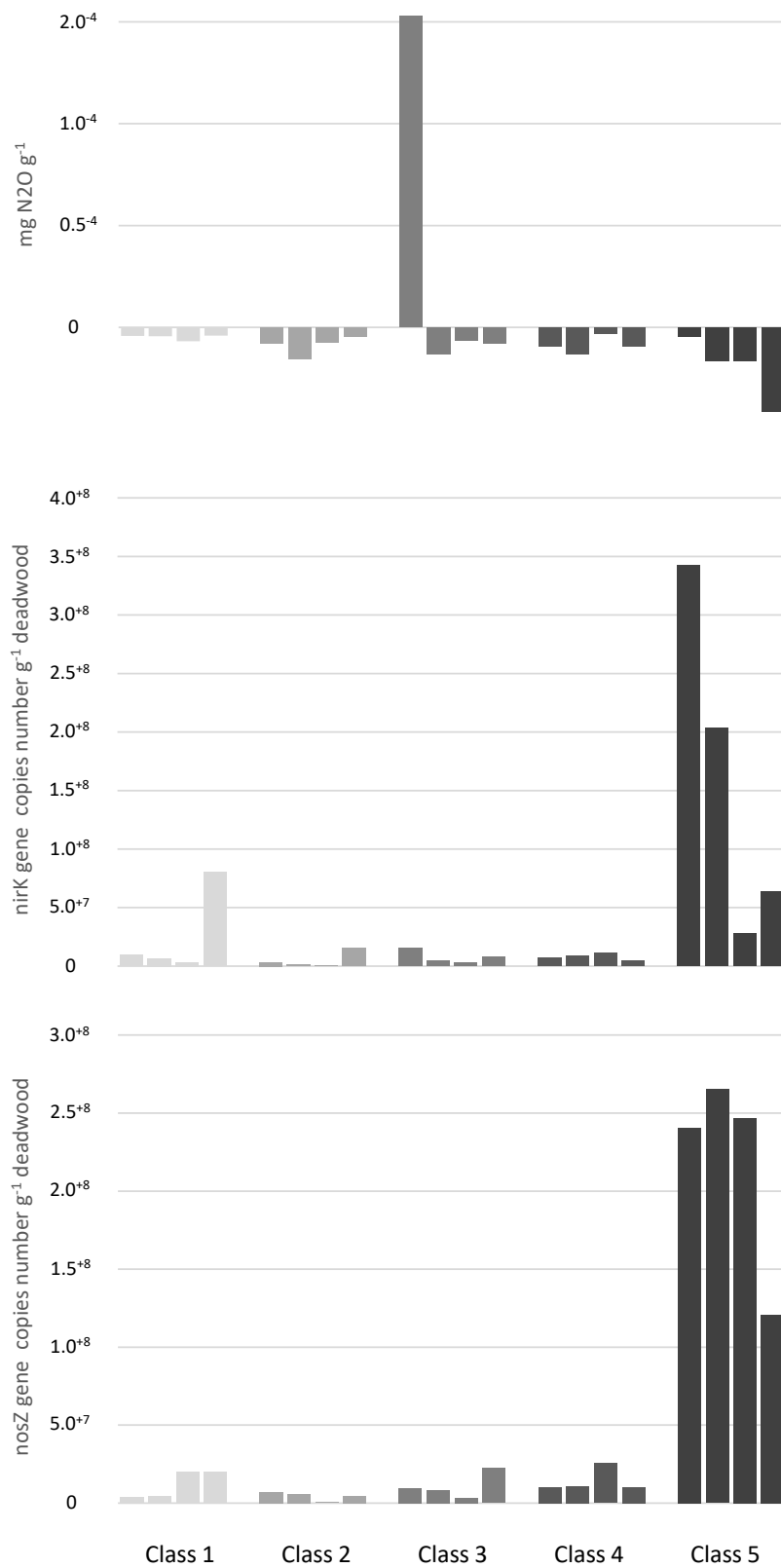


Figure S2. Maximum values of N₂O production (top) and nirK gene (middle) and nosZ gene (bottom) copies number (bottom) from each black pine deadwood sample.

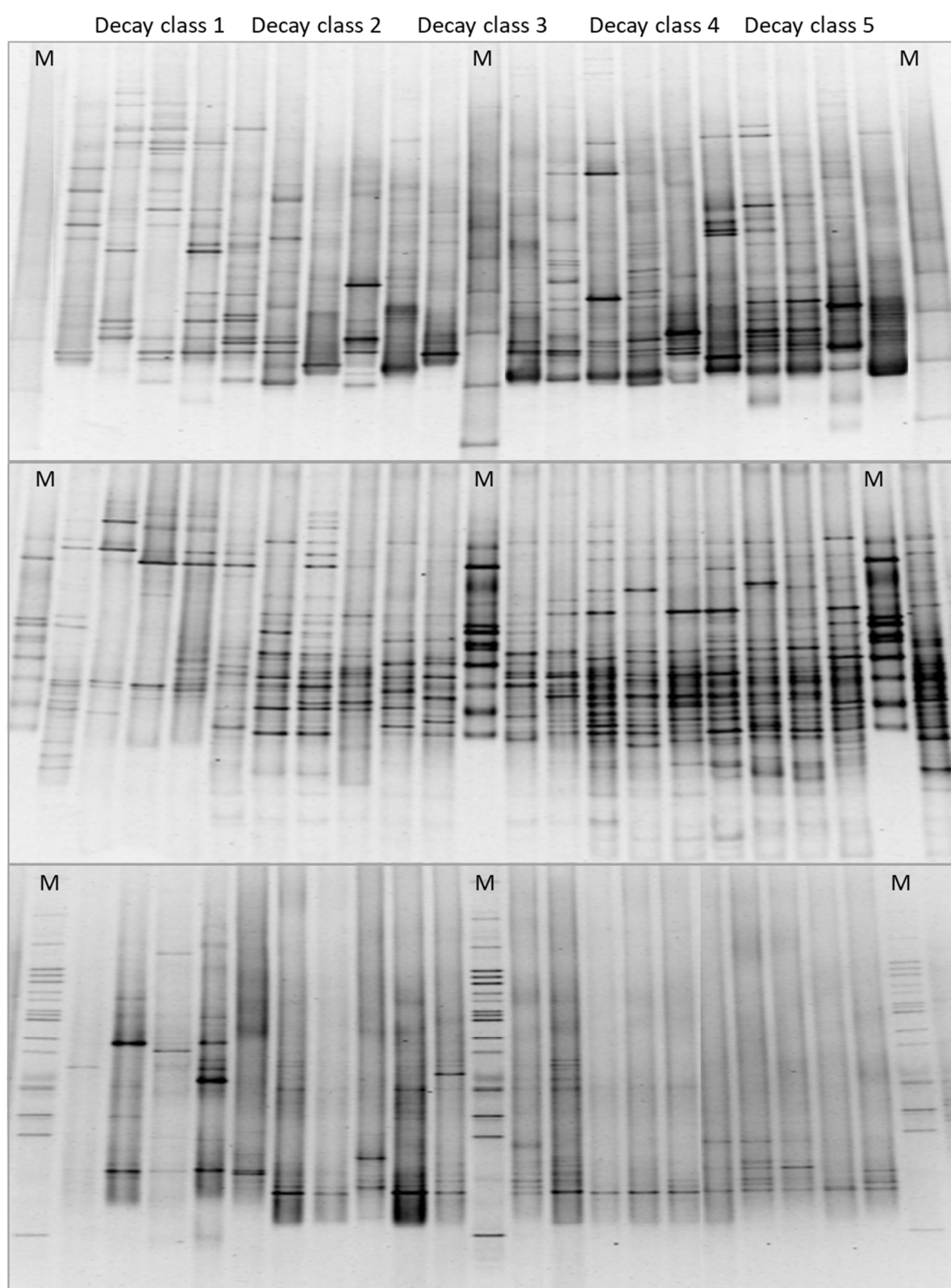


Figure S3. DGGE profiles of fungal 18S rDNA gene fragments (top), bacterial 16S rDNA gene fragments (middle), and actinobacterial 16S rDNA gene fragments (bottom). The letter M on the gel images indicates the marker used for normalization of bands.

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