

Supplemental material

Direct and indirect effects of land use on microbiomes of trap-nesting solitary bee larvae and nests

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Tables

Table SM1: Incorporated plots (Plot_ID) from the three Biodiversity Exploratories – Schorfheide-Chorin (SEG), Hainich-Dün (HEG), and Swabian Alb (AEG) within our research. Each plot is assigned a land-use intensity index (LUI) value derived from grazing (G), mowing (M), and fertilization (F) activities.

Plot_ID	G	M	F	LUI
AEG1	0	1.91	2.34	2.06
AEG2	0	2.87	9.31	3.49
AEG3	0	1.91	0	1.38
AEG4	1.24	0.96	1.6	1.95
AEG5	1.01	0.96	1.6	1.89
AEG6	0.76	0.96	0.81	1.59
AEG7	0.34	0	0	0.59
AEG8	0.4	0.96	0	1.16
AEG9	0.82	0	0	0.9
HEG1	0	1.91	10.24	3.49
HEG2	0.2	1.91	3.24	2.31
HEG3	0.2	1.91	3.24	2.31
HEG4	0	0.96	3.15	2.03
HEG5	0	1.91	2.46	2.09
HEG6	0.17	0.96	4.88	2.45
HEG7	5.13	0	0	2.26
HEG8	1.48	0	0	1.22
HEG9	0.58	0	0	0.76
SEG1	0	0.96	0	0.98
SEG2	1.17	0	0	1.08
SEG3	1.23	0.96	0	1.48
SEG4	0	0	0	0
SEG5	0	0.96	0	0.98
SEG6	3.04	0.96	0	2
SEG7	2.32	0	0	1.52
SEG8	3.76	0.96	0	2.17
SEG9	2.39	0	0	1.55

Table SM2: Information about sample size (N) of *Osmia bicornis* bee specimens (bee larvae & pupae) and nesting material like larval pollen provisions (pollen) and soil nest enclosures (soil) using 16S metabarcoding.

Bee species	Nesting material		Bee	
<i>Osmia bicornis</i>	Pollen (N)	Soil (N)	Larvae (N)	Pupae (N)
	66	34	24	20

Table SM3: Richness, diversity, and evenness of the microbiome of bee larvae, pupae, larval pollen provisions (pollen), and soil nest enclosures of *O. bicornis* bee nests. The given values represent means and their respective standard deviations (SD).

<i>Osmia bicornis</i>		Richness	Diversity	Evenness
	Larvae	416.95±146.24	3.13±0.76	0.52±0.12
	Pupae	368.11±136.53	3.46±0.33	0.60±0.08
	Pollen	434.37±193.99	2.27±0.79	0.38±0.13
	Soil	1418.03±450.73	5.01±0.93	0.69±0.11

Figures

Figure SM1: Artificial trap-nests, specifically designed to attract cavity-nesting solitary bee species, were installed at the wooden fence of the weather station in each plot (left) across the three Biodiversity Exploratories (right). Each trap nest was equipped with a plastic tube containing 60-80 hollow reed sticks, measuring approximately 20 cm long and 4-12 mm in width (middle). Monitoring of trap nests occurred at 8-10 week intervals from March to October to encompass the full flight period of trap-nesting solitary bees. Sample collection involved five sessions in 2017 and three sessions in 2018. Reed sticks found to have sealed entrances were replaced with fresh sticks and promptly transported to the University of Würzburg laboratory. The research plots were distributed geographically and situated in three different locations in Germany: the UNESCO Biosphere Reserve Swabian Alb (ALB) in southern Germany, the National Park Hainich-Dün (HAI) in central Germany, and the UNESCO Biosphere Reserve Schorfheide-Chorin (SCH) in the northeast of Germany. Bee collection activities were conducted under permits (ALB: AZ: 55-8/8848.02-07, HAI: AZ: 63.02/15.02.11-bio_expl2017.2 & AZ: 1011-17-301, SCH:AZ: 4743/128+5#69122/2018). Additionally, a schematic map depicting the layout of the Biodiversity Exploratory framework is provided for reference (right).

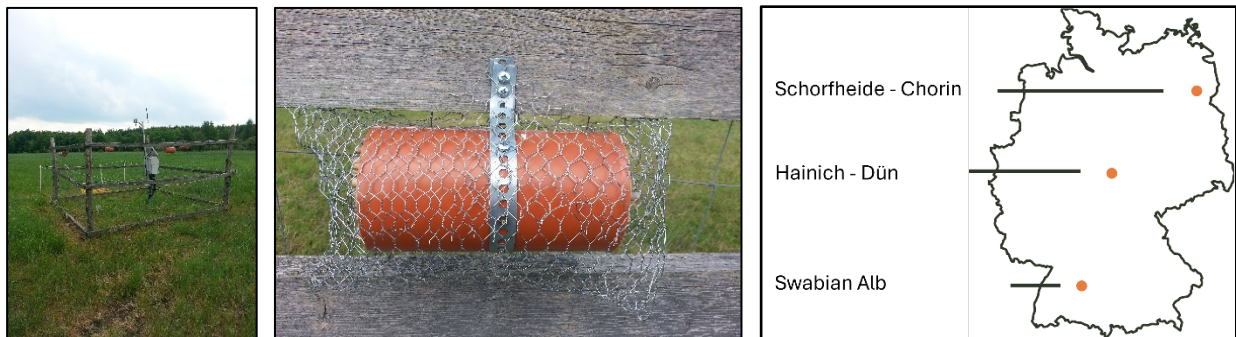


Figure SM2: Richness (left) and Shannon diversity (right) of microbial communities in different *O. bicornis* sample types (larval pollen provision, soil nest enclosure, larvae, and pupae) based on the number of ASVs.

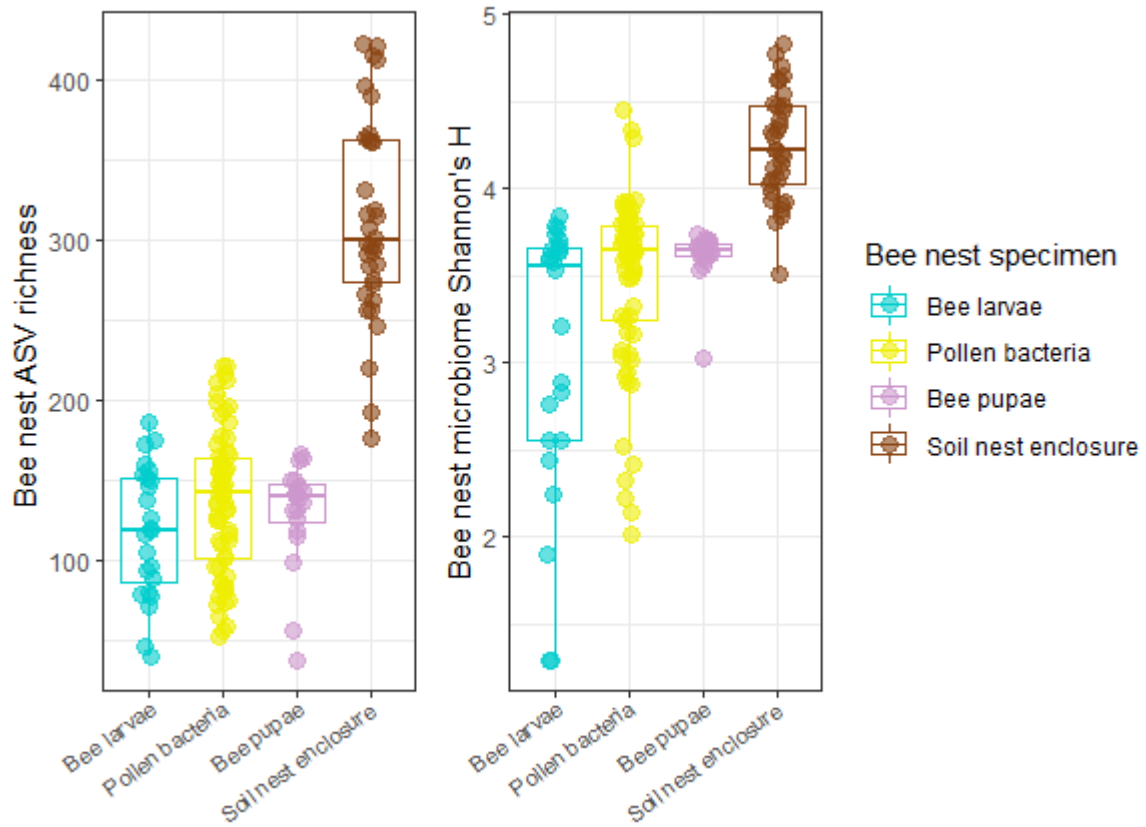


Figure SM3: Differences in bacterial compositions of *O. bicornis* sample types (larval pollen provisions, soil nest enclosures, bee larvae & bee pupae) (represented by different colors) by non-metrical multi-dimensional scaling (NMDS, stress=0.102) based on Bray-Curtis distances using transformed relative abundances data of ASV (amplicon sequent variants). ASVs were plotted for all bee nest specimens for all 27 plots. Each dot represents one sample of one nest ($n=144$)(Figure A). Variabilities in community structures of different bee species and specimen types. The analysis of Bray-Curtis distances as distances to group centroids of each community. Differences between sample types were assessed by multivariate analysis of group dispersions (betadisper/adonis)(FigureB).

Differences in community composition between bee nest specimens

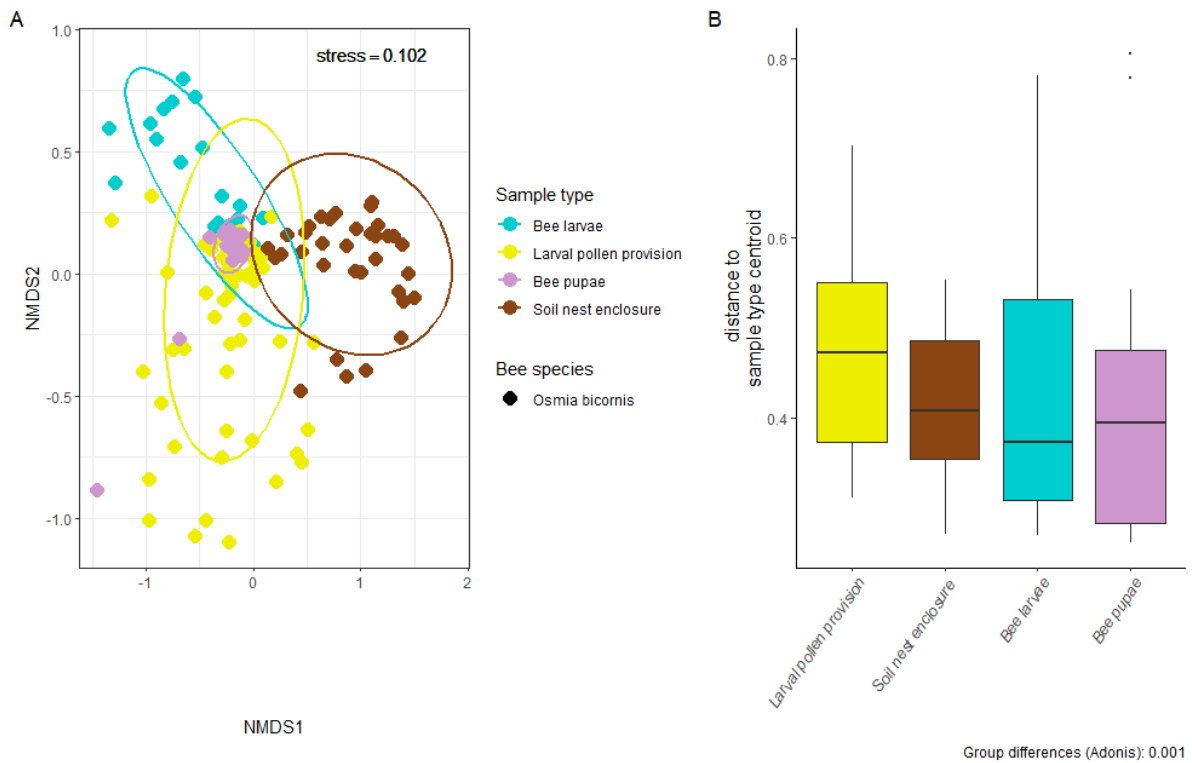


Figure SM4: Venn diagrams analysis of shared amplicon sequence variants (ASVs) among the twenty most abundant taxa across all investigated sample types of *O. bicornis* bee nests. The input ASV table represents presence/absence data, with occurrences of shared presence across bacterial communities of the different sample types. Specifically, 75 ASVs were found to be shared among all four sample types, including larval pollen provisions, bee larvae, bee pupae, and soil nest enclosures.

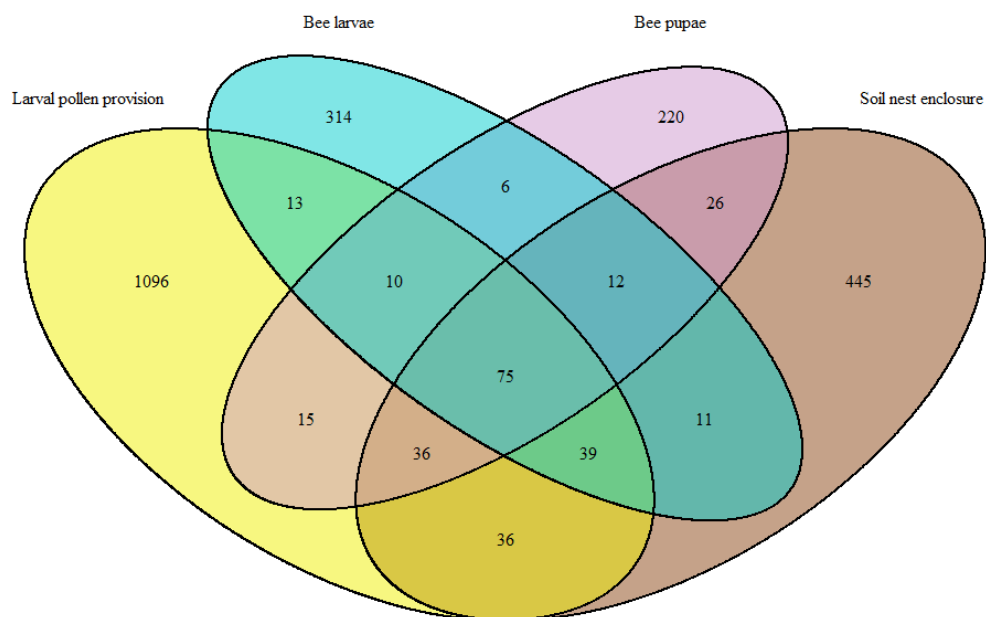


Figure SM5:Mean relative abundance of bacterial taxa (twenty most abundant taxa) of *O. bicornis* bee nest samples (larval pollen provision (N=66), soil nest enclosure (N=34), larvae (N=24), pupae (N=20)).

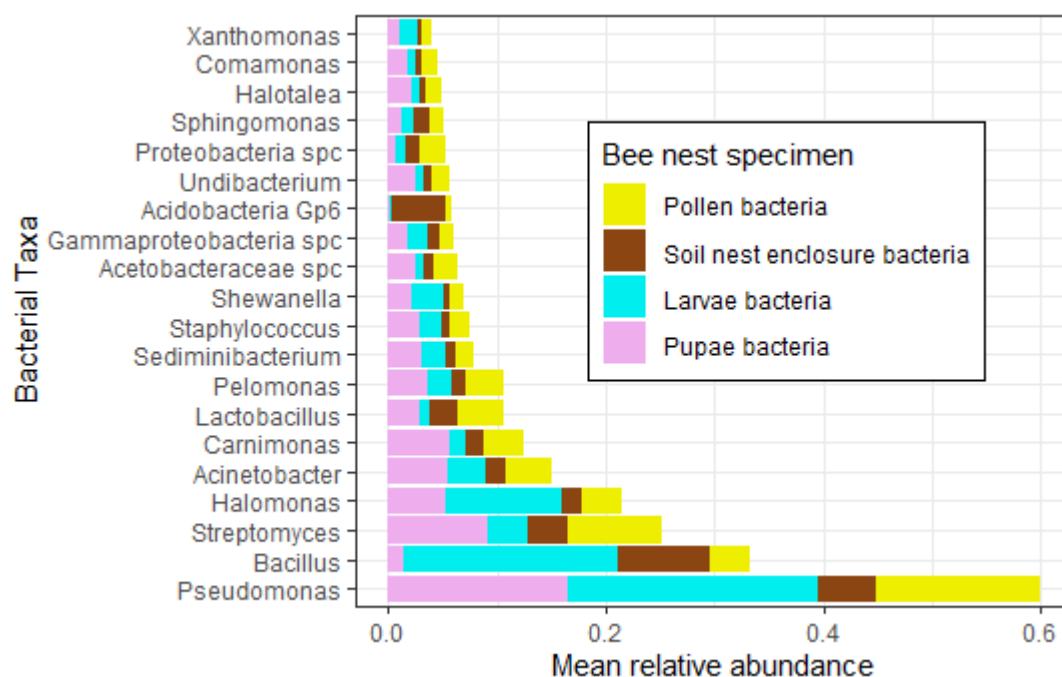


Figure SM6:Mean relative abundance of bacterial taxa (twenty most abundant taxa) of *O. bicornis* bee nest samples (larval pollen provision (=pollen), soil nest enclosure (=soil), larvae and pupae). according to different land-use intensities (Low:LUI<1.1, Intermediate:LUI 1.11-2.3,High:LUI>2.31)

Mean relative abundance of bacterial taxa in *Osmia bicornis* bee nest specimens

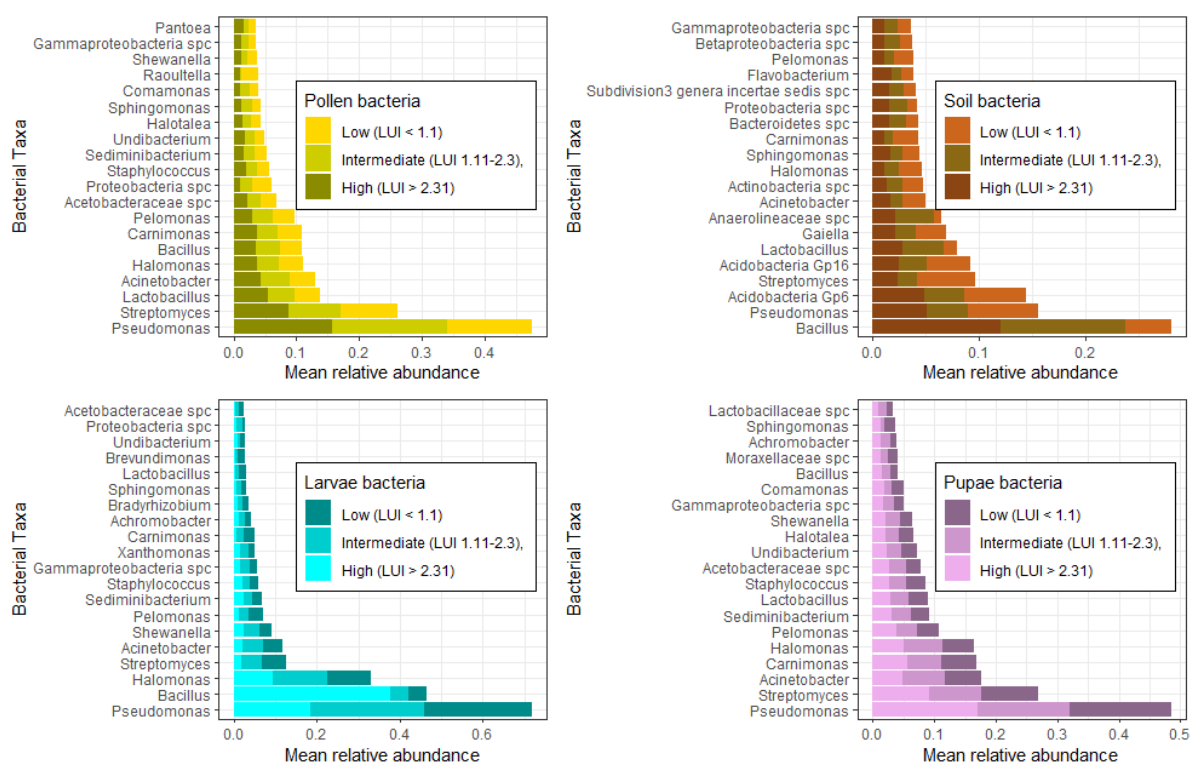


Figure SM7: Shannon diversity of different *O. bicornis* bee nest specimens (Bee larvae and pupae, pollen and soil nest enclosures) collected from nests installed at plots differing in land-use intensity in three biogeographical regions in Germany (Exploratories: Swabian Alb, Hainich-Dün and Schorfheide-Chorin) based on revealed ASVs (Amplicon sequent variants) per bee nest (Figure A). Variabilities in community structures of different land-use intensities (LUI categories: low-high) and *O. bicornis*. The analysis of Bray-Curtis distances represents the beta-diversity as distances to group centroids of each community. Differences between land-use categories within a specimen group (Figure B-E) were assessed by multivariate analysis of group dispersions (betadisper/adonis).

Impact of LUI on different *Osmia bicornis* bee nest specimens

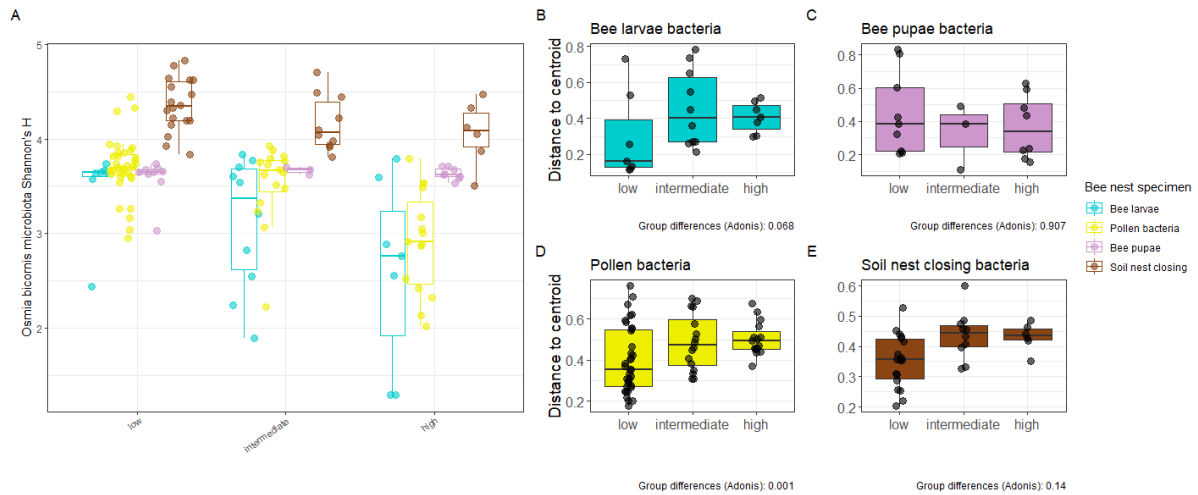


Figure SM8: Effects of Mowing (A), Grazing (B) and Fertilization (C) intensity on Shannon bacterial diversity of bee larvae & pupae, larval pollen provisions and soil nest enclosure of *O. bicornis* sampled from trap nests installed at plots differing in land-use intensity (LUI) in three biogeographical regions in Germany (Exploratories: Swabian Alb, Hainich-Dün and Schorfheide-Chorin). Shannon diversity is based on revealed ASVs (Amplicon sequent variants) per bee nest.

