

High-throughput amplicon sequencing of rRNA genes requires a copy number correction to accurately reflect the effects of management practices on soil nematode community structure

B. J. Darby, T. C. Todd, M. A. Herman

### Supplemental Methods:

#### *Details of Genetic Algorithm*

The genetic algorithm was run in MATLAB version 2012b (Mathworks, Natick, MA). We seeded a population of 20,000 individuals with 28 loci, each representing a theoretical relative copy number correction for the 28 families observed in the specimen-based counts. Each locus was assigned a value of 10.0 in the first generation. The fitness of each genotype was computed as the sum of squared errors (SSE) between the predicted community composition (relative abundance of copy-number adjusted sequence-based counts) and actual community composition (relative abundance of specimen-based counts):

$$SSE = \sum_{n=1}^N \sum_{i=1}^S (p_{i,n}^{seq} - p_{i,n}^{spec})^2$$

where  $n$  = sample,  $p_i$  = relative abundance of species  $i$  of sample  $n$  in the sequencing (" $p^{seq}$ ") or specimen (" $p^{spec}$ ") datasets,  $S$  = species richness,  $N$  = total number of samples.

In the first generation, all individuals in the population were equivalent (10.0 at each locus). In subsequent generations, individuals were ranked by fitness (SSE) and the 5,000 most fit individuals (lowest SSE) were kept for the next generation. Each parent also produced three daughters (mitotically, with no crossover) that were allowed to mutate at each loci by a random amount with a global average change of zero and a standard deviation of 5 (the minimum value at each locus was set to 1.0). Thus, each subsequent generation included the 5,000 most fit vectors from the previous generation and their 15,000 randomly mutated daughters. This was repeated for 10,000,000 generations and the genotype of the most fit individual after the final generation was selected as the optimized copy number solution.

**Supplementary Table S1.** Taxa identified by high-throughput amplicon sequencing of tallgrass prairie soil nematodes. Accession number, family identity, and species name are as listed in GenBank. Frequency is the number of occurrences that each accession was identified out of 62 field samples, and read count is the total number of reads that matched to a certain accession (from field samples only). The "Notes" column describes how some families were classified in the morphological enumerations (Table 2).

<u>Accession</u>	<u>Family</u>	<u>Notes</u>	<u>Species name</u>	<u>Frequency</u>	<u>Reads</u>
AY552975	Actinolaimidae	1	<i>Paractinolaimus</i> sp. PM-2002	13	296
AJ966514	Alaimidae	2	<i>Alaimus</i> sp. PDL-2005	1	6
AY284738	Alaimidae	2	<i>Alaimus parvus</i>	3	121
AF202151	Alloionematidae	3	<i>Rhabditophanes</i> sp. KR3	2	2
RA8	Alloionematidae	3	<i>Rhabditophanes</i> sp. RA8	3	30
AY552973	Anatonchidae	4	<i>Miconchus</i> cf. <i>fasciatus</i>	6	290
AY593913	Anguinidae		<i>Anguina tritici</i>	1	1
AY284635	Anguinidae		<i>Ditylenchus brevicaudatus</i>	25	553
AY284637	Anguinidae		<i>Ditylenchus</i> sp. 1JH-20	9	322
EU669909	Anguinidae		<i>Ditylenchus adasi</i>	4	115
HQ219210	Anguinidae		<i>Ditylenchus dipsaci</i>	1	14
AY284638	Anguinidae		<i>Pseudhalenchus minutus</i>	3	19
AY284640	Aphelenchidae		<i>Aphelenchus avenae</i>	20	687
AY284641	Aphelenchidae		<i>Aphelenchus</i> sp. JH-2004	1	1
AY284642	Aphelenchidae		<i>Paraphelenchus</i> sp. JH-2004	1	2
AY284643	Aphelenchoididae		<i>Aphelenchoides bicaudatus</i>	6	56
FJ040408	Aphelenchoididae		<i>Aphelenchoides saprophilus</i>	1	1
FJ520227	Aphelenchoididae		<i>Aphelenchoides fugianensis</i>	1	4
GU337995	Aphelenchoididae		<i>Aphelenchoides</i> sp. Be	6	51
AJ875153	Aporcelaimidae		<i>Aporcelaimellus</i> sp. F2	3	236
AJ875155	Aporcelaimidae		<i>Aporcelaimellus</i> sp. F5	27	2,035
AY284812	Aporcelaimidae		<i>Aporcelaimellus</i> cf. <i>paraobtusicaudatus</i>	3	210
AY284821	Belondiridae		<i>Dorylaimellus montenegricus</i>	3	13
AY552969	Belondiridae		<i>Dorylaimellus virginianus</i>	24	845
AY284825	Belondiridae		<i>Oxydirus oxycephalus</i>	2	2
EU130838	Belonolaimidae		<i>Belonolaimus longicaudatus</i>	2	3
EU368587	Belonolaimidae		<i>Tylenchorhynchus claytoni</i>	5	59
AY552965	Campydoridae	1	<i>Campydora demonstrans</i>	5	93
AF202148	Cephalobidae		<i>Acrobeles ciliatus</i>	11	99
AY284673	Cephalobidae		<i>Acrobeloides apiculatus</i>	12	6,532
AF202158	Cephalobidae		<i>Cephalobus</i> sp. PS1143	27	802
AF202160	Cephalobidae		<i>Cephalobus</i> sp. PS114852	7	13
HQ130507	Cephalobidae		<i>Chiloplacus</i> sp. KS584	7	184
AY284666	Cephalobidae		<i>Eucephalobus striatus</i>	5	40

AY284667	Cephalobidae		<i>Eucephalobus striatus</i>	1	34
AF202150	Cephalobidae		<i>Pseudacrobeles variabilis</i>	26	902
AY284716	Choanolaimidae	1	<i>Choanolaimus psammophilus</i>	32	2,149
AJ920337	Cloacinidae	2	<i>Labiostromyulus bipapillosus</i>	1	27
AJ966480	Criconematidae		<i>Criconema</i> sp. PDL-2005	1	3
AY284626	Criconematidae		<i>Mesocriconema xenoplax</i>	33	1674
AY593940	Cyatholaimidae	2	<i>Achromadora</i> cf. <i>terricola</i>	5	39
AY552968	Diphtherophoridae		<i>Diphtherophora obesus</i>	7	703
AY284777	Dorylaimidae	5	<i>Dorylaimus stagnalis</i>	4	5
AJ966489	Dorylaimidae	5	<i>Mesodorylaimus japonicus</i>	25	7,552
AJ966513	Dorylaimidae	6	<i>Wilsonema schuurmansstekhoveni</i>	20	454
AY593917	Ecphyadophoridae	7	<i>Ecphyadophora</i> sp. JH-2004	1	1
AJ920351	Haemonchidae	2	<i>Ostertagia leptospicularis</i>	1	69
AJ966486	Hoplolaimidae		<i>Helicotylenchus dihystra</i>	12	1,089
AY284606	Hoplolaimidae		<i>Helicotylenchus pseudorobustus</i>	40	6,281
EU306354	Hoplolaimidae		<i>Helicotylenchus varicaudatus</i>	9	291
FJ969124	Hoplolaimidae		<i>Helicotylenchus multicinctus</i>	1	1
AY552970	Ironidae	2	<i>Ironus</i> sp. 2-PM-2004	1	5
AY284831	Leptonchidae		<i>Leptonchus granulatus</i>	1	1
AY284837	Leptonchidae		<i>Tylencholaimellus striatus</i>	2	19
HM921344	Longidoridae		<i>Xiphinema rivesi</i>	34	4,174
AF442194	Meloidogynidae		<i>Meloidogyne hapla</i>	1	1
EF189177	Meloidogynidae		<i>Meloidogyne spartinae</i>	10	81
EU669946	Meloidogynidae		<i>Meloidogyne ardenensis</i>	9	86
AY284721	Microlaimidae	6	<i>Prodesmodora circulata</i>	1	1
AY593937	Monhysteridae	2	<i>Eumonhystera filiformis</i>	3	12
EF591334	Monhysteridae	2	<i>Geomonhystera villosa</i>	3	6
FJ040465	Monhysteridae	2	<i>Geomonhystera</i> sp. 1998	1	15
AY284749	Mononchidae		<i>Clarkus papillatus</i>	7	118
AY552966	Mononchidae		<i>Clarkus papillatus</i>	3	4
AY284767	Mononchidae		<i>Coomansus parvus</i>	17	2,175
AY284745	Mononchidae		<i>Prionchulus muscorum</i>	1	2
AY593950	Mydonomidae	1	<i>Dorylaimoides limnophilus</i>	9	104
AY593951	Mydonomidae	1	<i>Dorylaimoides</i> sp. JH-2003	1	1
AB361437	Mylonchulidae	4	<i>Mylonchulus brachyuri</i>	7	103
AB361441	Mylonchulidae	4	<i>Mylonchulus hawaiiensis</i>	4	271
AB361447	Mylonchulidae	4	<i>Mylonchulus sigmaturus</i>	2	2
AY284760	Mylonchulidae	4	<i>Mylonchulus</i> sp. JH-2004	10	228
FJ040445	Neodiplogasteridae	2	<i>Pristionchus americanus</i>	1	4
RA4	Neodiplogasteridae	2	<i>Pristionchus pseudaeerivorous</i>	27	5,382
EU477379	Nordiidae	1	<i>Enchodelus veletensis</i>	4	18
AY284788	Nordiidae	1	<i>Pungentus silvestris</i>	20	329

AY552963	Nygolaimidae	1	<i>Aquatides christei</i>	2	4
FJ969131	Oxystominidae	2	<i>Odontolaimus</i> sp. OdLaSp1	14	234
AJ567385	Panagrolaimidae		<i>Panagrolaimus davidi</i>	6	31
GU014546	Panagrolaimidae		<i>Panagrolaimus detritophagus</i>	16	1,301
U81579	Panagrolaimidae		<i>Panagrolaimus</i> sp.	1	1
AY284634	Paratylenchidae		<i>Paratylenchus</i> cf. <i>neoamblicephalus</i>	24	1,594
AJ966473	Plectidae		<i>Anaplectus</i> sp. PDL-2005	18	598
FJ474097	Plectidae		<i>Ceratoplectus</i> cf. <i>assimilis</i>	4	6
AF036602	Plectidae		<i>Plectus aquatilis</i>	23	726
AY284703	Plectidae		<i>Plectus</i> cf. <i>parietinus</i>	7	162
FJ969135	Plectidae		<i>Plectus tenuis</i>	18	644
AF202155	Plectidae		<i>Tylocephalus auriculatus</i>	36	1,872
EU669927	Pratylenchidae		<i>Pratylenchus scribneri</i>	5	22
EU669958	Pratylenchidae		<i>Pratylenchus scribneri</i>	9	282
AF036603	Prismatolaimidae		<i>Prismatolaimus intermedius</i>	13	276
AY284728	Prismatolaimidae		<i>Prismatolaimus</i> cf. <i>dolichurus</i>	2	2
AY593957	Prismatolaimidae		<i>Prismatolaimus dolichurus</i>	8	84
AJ966472	Qudsianematidae		<i>Allodorylaimus</i> sp. PDL-2005	3	274
AY284779	Qudsianematidae		<i>Chrysonema attenuatum</i>	1	1
EF207245	Qudsianematidae		<i>Chrysonema attenuatum</i>	22	11,307
AY284783	Qudsianematidae		<i>Ecumenicus monohystera</i>	20	850
AY284800	Qudsianematidae		<i>Eudorylaimus</i> sp. JH-2004	1	13
AY284807	Qudsianematidae		<i>Labronema vulvapapillatum</i>	2	11
AY552972	Qudsianematidae		<i>Labronema ferox</i>	3	69
AJ966492	Qudsianematidae		<i>Microdorylaimus</i> sp. PDL-2005	3	97
AY284804	Qudsianematidae		<i>Microdorylaimus miser</i>	3	40
AY284778	Qudsianematidae		<i>Prodorylaimus uliginosus</i>	22	402
AY284794	Qudsianematidae		<i>Thonus minutus</i>	1	71
AY284797	Qudsianematidae		<i>Thonus</i> sp. JH-2004	5	306
AY284660	Rhabditidae		<i>Mesorhabditis</i> sp. JH-2004	51	56,902
HQ130505	Rhabditidae		<i>Mesorhabditis</i> sp. KS601	6	8
MR2	Rhabditidae		<i>Mesorhabditis</i> sp. KS602	7	9
EU196009	Rhabditidae		<i>Oscheius tipulae</i>	56	48,689
HQ130503	Rhabditidae		<i>Oscheius</i> sp. FVV-2	48	11,650
HQ130504	Rhabditidae		<i>Rhabditis</i> sp. KS594	58	218,198
AY284710	Rhabdolaimidae	2	<i>Rhabdolaimus</i> cf. <i>terrestris</i>	3	4
FJ969139	Rhabdolaimidae	2	<i>Rhabdolaimus aquaticus</i>	9	199
FJ040422	Steinernematidae	2	<i>Steinernema glaseri</i>	2	104
EF197730	Tripylidae	2	<i>Tripyla</i> sp. SAN-2007b	1	4
FJ480407	Tripylidae	2	<i>Tripylina tearoha</i>	6	1,305
FJ969113	Tylenchidae		<i>Aglenchus agricola</i>	16	237
EU130839	Tylenchidae		<i>Basiria gracilis</i>	8	224

AY593915	Tylenchidae	<i>Boleodorus thylactus</i>	8	76
AY284583	Tylenchidae	<i>Coslenchus franklinae</i>	1	1
AY284591	Tylenchidae	<i>Filenchus thornei</i>	16	397
AY284584	Tylenchidae	<i>Lelenchus leptosoma</i>	6	105
FJ969129	Tylenchidae	<i>Miculenchus salvus</i>	11	203
AY284590	Tylenchidae	<i>Ottolenchus discrepans</i>	5	11
EU130840	Tylenchidae	<i>Psilenchus</i> sp. CA12	9	56
EU306348	Tylenchidae	<i>Tylenchus arcuatus</i>	3	9
AY284833	Tylencholaimidae	<i>Tylencholaimus</i> sp.	3	12
AY552978	Tylencholaimidae	<i>Tylencholaimellus affinis</i>	2	5
EF207253	Tylencholaimidae	<i>Tylencholaimus mirabilis</i>	11	67
	Total Accessions:	129	Total Reads:	407,908
	Non-singletons:	117	Rhabditidae:	335,456

## Notes:

1. Classified as "Misc. Dorylaimida".
2. Classified as "Misc. bacterivores"
3. Classified as "Rhabditidae"
4. Classified as "Mononchidae"
5. Classified as "Qudsianematidae"
6. Classified as "Plectidae"
7. Classified as "Misc. Tylenchidae"
8. Classified as "Tylencholaimidae"

**Supplementary Table S2.** Summary of linear mixed model analysis performed on Specimen Counts, sequence-based Read Counts, and copy-number corrected Virtual Specimens. Columns depict significant effects ( $p < 0.05$ ) of burning ("B"), nitrogen ("N"), season ("S") plus all two way interactions.

<u>Family</u>	<u>Specimen Counts</u>	<u>Read Counts</u>	<u>Virtual Specimens</u>
Anguinidae		S	S, B
Aphelenchidae		S	N
Aphelenchoididae	S*N	S*N	S*B, S*N, B*N
Aporcelaimellidae	B, N	S	
Belondiridae	B, N, S*N	B	B, N, S*N
Cephalobidae		S	
Criconematidae	B, N		B
Diphtherophoridae	S		
Hoplolaimidae	B, S*B	S	S, B
Leptonchidae	S		
Longidoridae		S	N
Meloidogynidae	S, B		S
Mononchidae	B, S*N		B
Panagrolaimidae	S*N	S	S, B, S*N, N
Paratylenchidae	B	B, S	S, B, N
Plectidae	S	S	
Pratylenchidae	S, B, S*B, N		
Prismatolaimidae	B		B
Qudsianematidae		B*N, S	S, B
Rhabditidae	B, N	S	S
Telotylenchidae	S, B		
Tylenchidae	B		
Tylencholaimidae	S, B, N, S*N		B, N

**Supplementary Table S3.** Principal Components Analysis eigenvectors for families from the specimen-based counts, sequence-based read counts, and copy number-corrected virtual nematode counts. Columns indicate the PCA eigenvector loading weight from principal component 1 ("Prin1") and principal component 2 ("Prin2") from the Specimen Counts, Read Counts, and from the Virtual Specimen counts (sequence-based data corrected for copy number factor).

<u>Family</u>	<u>Specimen Counts</u>		<u>Read Counts</u>		<u>Virtual Specimens</u>	
	<u>Prin1</u>	<u>Prin2</u>	<u>Prin1</u>	<u>Prin2</u>	<u>Prin1</u>	<u>Prin2</u>
Anguinidae	0.06	0.12	0.25	-0.12	0.27	-0.06
Aphelenchidae	0.17	0.18	0.21	-0.19	0.19	-0.32
Aphelenchoididae	-0.05	-0.03	0.18	0.34	0.06	0.18
Aporcelaimellidae	0.26	-0.17	0.22	-0.13	0.27	0.04
Belonidiridae	0.33	-0.17	0.18	0.23	0.19	0.28
Cephalobidae	0.09	0.42	0.22	-0.15	0.19	-0.21
Criconematidae	0.30	-0.10	0.26	0.11	0.30	0.20
Diphtherophoridae	-0.05	0.10	0.10	-0.14	0.05	-0.07
Hoplolaimidae	0.19	0.24	0.27	-0.07	0.26	-0.16
Leptonchidae	0.16	0.13	0.01	0.00	-0.07	0.18
Longidoridae	0.02	0.34	0.23	-0.15	0.21	-0.25
Meloidogynidae	0.28	-0.11	0.08	0.51	0.09	0.25
Mononchidae	0.18	-0.10	0.24	-0.04	0.26	0.14
Panagrolaimidae	-0.14	0.28	0.19	0.15	0.16	-0.16
Paratylenchidae	0.26	0.22	0.14	-0.38	0.10	-0.35
Plectidae	0.09	0.27	0.24	-0.15	0.21	0.05
Pratylenchidae	0.12	0.24	0.17	0.16	0.22	0.01
Prismatolaimidae	0.24	0.20	0.24	0.13	0.27	0.02
Qudsianematidae	0.25	-0.03	0.21	-0.03	0.17	0.28
Rhabditidae	-0.17	0.35	-0.18	0.15	-0.30	0.14
Telotylenchidae	0.19	0.12	0.11	-0.06	0.13	-0.01
Tylenchidae	0.36	0.05	0.23	0.10	0.25	0.12
Tylencholaimidae	0.15	-0.13	0.21	0.38	0.16	0.20

**Supplementary Table S4.** Summary of linear mixed model analysis on trophic group abundance of virtual specimen data. A) F-values on  $\log(x+1)$ -transformed virtual specimen counts, with abundance of herbivores (HERB), fungivores (FUNG), bacterivores (BACT) or predator/omnivores (PRED/OMNI) as a dependant variable. B) Least-Squares Means for Burn vs. Non-burned treatment. C) LS Means for Ambient vs. Nitrogen-enriched treatments.

A)	<u>Effect</u>	<u>df</u>	<u>HERB</u>	<u>FUNG</u>	<u>BACT</u>	<u>PRED/OMNI</u>
	Season	1,8	13.81***	2.88	20.95***	19.13***
	Burn	2,8	18.16***	4.12*	4.5*	14.00***
	B × S	2,8	2.54	0.13	2.14	4.90*
	Nitrogen	1,8	9.09**	0.00	0.01	0.31
	N * S	2,8	2.39	0.50	3.67	0.24
	B × N	2,8	1.64	0.20	0.82	0.44
B)	<u>Burn means</u>		<u>HERB</u>	<u>FUNG</u>	<u>BACT</u>	<u>PRED/OMNI</u>
	Non-Burned		38.8 <sup>A</sup>	15.9 <sup>A</sup>	162.1 <sup>A</sup>	34.6 <sup>A</sup>
	Burned		194.8 <sup>B</sup>	45.9 <sup>B</sup>	228.9 <sup>B</sup>	117.7 <sup>B</sup>
C)	<u>Nitrogen means</u>		<u>HERB</u>	<u>FUNG</u>	<u>BACT</u>	<u>PRED/OMNI</u>
	Ambient		49.1 <sup>A</sup>	26.8	190.1	70.1
	N-enriched		154.3 <sup>B</sup>	27.5	194.6	58.4

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .