Canoco 5 – a short introduction

Software for multivariable data analysis and visualization Canoco shows it!

Cajo J.F. ter Braak and Petr Šmilauer







Ex1: Comparison of microbiota among three groups Unconstrained (PCA)



Feces transplant study: van Nood et al. 2013 NEJM Data: **microbiota taxa** (Susana Fuentes, W. de Vos)



Ex2: Comparison among two groups (1)



VAGENINGEN UR For quality of life Constrained (RDA)

- Extension of ttest
- Horizontal (constrained) axis
 difference of Control and Colic
- Vertical (unconstrained) axis = main residual pattern
- Correlation with Crying of babies

De Weerth et al 2012, Pediatrics Microbiota (Susana Fuentes, W. de Vos)

Ex2: Comparison among two groups (2)

We see three types of data in this example

- Response data (the main/focal data :
 - Amounts of 33 microbiota taxa
- Explanatory data:
 - Treatment, a factor with 2 levels (Control and Colic)
- Supplementary data:
 - Crying



Roles of data tables

Response data (main data table)

- to be visualized, perhaps in combination with others
- Supplementary data
 - to interpret the response data
- Explanatory data
 - to explain the response data
- Covariate data (for advanced users)
 - to account or adjust for.
 - to enable detection of structure in response after accounting for the variation explained by these covariates



Research questions and methods in Canoco

Derive patterns and relationships from data

- From field or laboratory
- From designed experiments or surveys
- Many noisy variables, non-linear relationships

Key methods

AGENI

- 1. Dimension reduction (ordination, factor analysis, multidimensional scaling)
- 2. Regression analysis, also non-linear
- 3. Combination of 1 and 2 (constrained ordination)
- 4. Visualization of results
- 5. Statistical testing by permutation



Starting a new Canoco project (1)

Canoco 5 focuses on research questions on a set of data

- A Canoco 5 project thus consists of
 - one or more data tables
 - analyses on these data

Easiest to start a new project with File Import project from Excel... (Alt-F-I-Enter)



🔓 Canoco 5		
File Edit Window	Help	
New project		- 🛃 🔁 🖽 🔘 🛃 🗛 🗦 🧨 A
📄 Open project		ut open
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🖬 Close project		😫 from Canoco 4.x files
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Starting a new Canoco project (2)



Starting a new Canoco project (3a)

Give names to YOUR units and variables

- choose from list or
- start typing
 - singular, then
 - plural

Import From Excel Files	s: Table 1/2	
Microsoft Excel - Dune_env	Table specified here will beco	me the main (primary) data table of this project.
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Starting a new Canoco project (3b)

Give names to YOUR units and variables

- Empty cells: 0 or mis Data kind is
- General or
- Compositional:
- -row sum has meaning

-variables measured on the same scale

The right choice helps to select suitable methods



Import From Excel Files: Table 1/2

A1

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B3 A

Sample 1

Sample 2 Sample 3

Sample 4

Sample 5

Sample

10 Sample 9

Labels

Sample

Sample 2

Sample 3

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Sample 5

Sample 6

Sample 2

Sample 8

Sample 9

Spec

R1

R2

R3

R4

R5

R6

R7

R8

R9



X

Starting a new Canoco project (5)

Result: two project data tables (Plants and Environment) and offer for starting analysis

Data tables:

you can

- View
- Edit
- Copy
- Export

Change kind/name etc.



		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	
	Labels	Achillea mi	Agrostis st	Aira praeco	Alopecurus	Anthoxanth	Bellis perei	Bromus ho	Chenopodi	Cirsium arv	Eleocharis	;
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R4	4		8		2		2	3		2		T
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[R22]			V	c	No	Help						
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[R24]												

Starting a new Canoco project (6)

Accepting the offer and all default choices leads to

-Summary of DCA analysis

-Two graphs

Save your project!

File Save.. or

Ctrl-S



G Canoco 5 - [Analysis Unconstrained-supp	pl-vars]									
G File Edit Project Data Analysis Graph Window Help										
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Add table Delete table	Method: DCA with supplementary variables									
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Starting a new Canoco project (6)

Accepting the offer and all default choices leads to

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To view the data again.	click	Plants		Summary of Results					
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Eilo Savo or				Eigenvalues		0.5360	0.2869	0.0814	0.0481
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Inspecting a graph with Describe Contents

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All scores are available too:

Edit | *Settings* | *Canoco5 Options:*

Click I on the toolbar
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Summary	Log	Cases	RespVar	s Supply	Vars 0	Graph 1	Graph 2						
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Chenopodiu	ım alt	oum	3.0359	1.3324	-2.3487	-1.8633	1.0000	1.0000	0.4963	0.0526	2.8354	1.8633	171.4669
Cirsium arv	ense		2.1552	2.0161	-4.5330	2.2143	2.0000	1.0000	0.1464	0.9517	4.5330	1.7246	247.2326

• Click again to hide the score tabs



Adding a new analysis to the project (1)

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l lab	Delete	4
	Close analysis notebooks	8
	R5 5	2
New Modify Re-analyze		
Hid Create new analysis in this project		

Adding a new analysis to the project (2)

Select:

1. Tables



Select data tables that can participate in suggested analyses

- Plants (plant species composition of meadows)
- Environment (environmental variable values of meadows)

Canoco Adviser: Create New Analysis

2. Focal table



Select table focal for the new analysis:

Plants (plant species composition of meadows) Environment (environmental variable values of meadows)

Canoco Adviser: Create New Analysis

3. Template for analysis





Select the analysis to be created:

Adding a new analysis to the project (3)

3. Select template

-double click on bold terms to fold/unfold

(Can enlarge dialog window to see all) Canoco Adviser: Create New Analysis



Select the analysis to be created:

	 Standard Analyses Compare-constrained-unconstrained (plant species ~ environmental variable) 								
	 Constrained (plant species ~ environmental variables) Interactive-forward-selection (plant species ~ environmental variables) Test-constr-axes (plant species ~ environmental variables) Unconstrained (plant species) Unconstrained-suppl-vars (plant species ~ [environmental variables] *) 								
T	 Variation Partitioning Analyses Advanced Constrained Analyses Specialized Analyses Handcrafted Analysis Create customized 	Alphabetic list of templates							
	Import Canoco4 .CON file	4							
	In the selected analysis, you would								
	summarize the part of the variation in by environmental variables	plant species composition explained 🔺							

Canoco Adviser suggests these analyses based on the tables selected earlier. In addition to Adviser suggestions, you can create an analysis manually or import it from Canoco 4.5 CON file. Both possibilities are under the heading Handcrafted Analyses.

Help

< Back

Finish

Cancel



Adding a new analysis to the project (4)

Standard analyses:

- Constrained: response variables ~ predictors
- Unconstrained: response variables

response variables ~ [supplementary variables]

- Compare constrained unconstrained
- Test constrained axes
- Interactive forward selection of predictors
 - See also: Summarize effects of expl variables

See Advanced ... for constrained analysis with covariates



Adding a new analysis to the project (5)

Standard analyses:

Ordination method

Method Linear Unconstrained © PCA Constrained © RDA

Unimodal

CA (DCA)
CCA

PCA: Principal component analysis

RDA: Redundancy analysis

CA (DCA): Correspondence analysis(Detrended)

CCA: Canonical correspondence analysis



Adding a new analysis to the project (6)

Test or Explore Predictor Effects						
 Not performed All constrained axes test First constrained axis test Both above tests performed 	 Forward selection of expl. variables Summarize effects of expl. variables 					
Permutation Test Parameters						
 Unrestricted permutations Time series or linear transects Rectangular grids Hierarchical design Read from file 	 Number of permutations: 499 Random number generator seeds: 23239 and 945 Randomize Blocks defined by covariates Leverage correction of residuals 					
Disable random shifts from mirror						



Summarize effects of expl. variables.

Dune meadow data

Plant species ~ Environment (CCA)

Term Effects							Term Effects		
P values correction Simple Effects	n: False disc	overy rate	•				P values correction: Simple Effects	False discovery rate	
Name Moisture Management.NM Manure Amount	Explains % 19.4 15.0 11.3	pseudo-F 4.3 3.2 2.3	P 0.002 0.004 0.016	P(adj) 0.016 0.016 0.04267	Сору	y	Name E Moisture	False discovery rate Holm correction Bonferroni correction	P 2 (
Conditional Effects	5								
Name Moisture Management.NM A1 horizont	Explains % 19.4 12.2 6.7	pseudo-F 4.3 3.0 1.7	P 0.002 0.002 0.06	P(adj) 0.008 0.008 0.16	Сору	y			



Forward selection of expl. variables

- Color code for significance
- FDR testing on-line, but only for viewed variables
 - Tip: increase window size to get correct FDR



Forward Selection Step									
Candidate Terms									
Name	Contribution %	F	Р	P(adj)					
Moisture	34.9	4.3	0.002	0.016					
Management.NM	27.0	3.2	0.004	0.016					
Manure Amount	20.3	2.3	0.016	0.04267					
A1 horizont	19.1	2.1	0.032	0.064					
Management.SF	16.7	1.8	0.058	0.0928					
Management.HF	12.5	1.4	0.17	0.22667					
Management.BF	11.9	1.3	0.228	0.26057					
Use Type	10.9	1.2	0.294	0.294					
Test	Include		Stop		Help				
Include whole	factor								
Term Contribution									
All considered vari	ables explain tog	ether : d term	0 would a	f total vari contribute	ation 34 0%				
	in, the highlighte	u term	would (contribute .	J-1.570				
Selected Terms									
Order Name P	P(adj)								
P values correction	: False discovery	/ rate	-						

Canoco Adviser

On the basis of the data properties the Adviser suggests

Transformation and standardization of variables

right-click on top-left cell in data sheet

Or use

Data | Default transformation and ...

C5 C6 [C7] [C8] Manure Amount [PO4]					
Manure Amount [PO4] 4 1000 2 100 4 10 4 10 2 923 Canoco5 Log transformation is suggested by Canoco Adviser for 1 environmental variable. After you close the Variable Transformations dialog, suggested transformations will be stored and used in the analyses. Do not show this dialog again	C5	C6	[C7]	[C8]	
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4 10 4 1 2 923 Canoco5 Log transformation is suggested by Canoco Adviser for 1 environmental variable. After you close the Variable Transformations dialog, suggested transformations will be stored and used in the analyses. Do not show this dialog again	2	100			
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2 923 Canoco5 Log transformation is suggested by Canoco Adviser for 1 environmental variable. After you close the Variable Transformations dialog, suggested transformations will be stored and used in the analyses. Do not show this dialog again	4	1			
Canoco5 Log transformation is suggested by Canoco Adviser for 1 environmental variable. After you close the Variable Transformations dialog, suggested transformations will be stored and used in the analyses. Do not show this dialog again	2	923			
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Canoco Adviser

On the basis of the data properties the Adviser suggests

- Transformation and standardization of variables
- Common analyses via templates
- Choice between Linear and Unimodal

Ordination mot	hod	
	nivu	
Method	Linear	Unimodal
Unconstrained	O PCA	CA (DCA)
Constrained	© RDA	OCA
Response data a	are compositional and have a gradier	at 3.7 SD units long

Re-advise

Response data are compositional and have a gradient 3.7 SD units long, so unimodal method is suggested, but linear one would be also a good choice



Methods in Canoco 5

Standard multivariate methods such as [partial] PCA/RDA/CA/DCA/CCA

But also:

- Response curves (regression by GLM/GAMs with one predictor)
- Contour plots (GLM/GAM with two predictors)
- Distance-based methods incl. nonmetric multidimensional scaling (NMDS)
- Variation partitioning
- Principal response curves (PRC)
- Generalized linear models (GLM) with permutation tests

Double-constrained analyses (dc-CA and dc-PCA)



and more...

Nonlinear response curves via GLM or GAM



GAMs or GLMs with two predictors





Distance-based methods eg. NMDS

E.g. from intercity train-time to a map of cities

PCO/NMDS/db-RDA/Procrustes analysis

Analysis Setup Wiza



	_
rd: NMDS Options (1)	}
Setup Non-metric MultiDimensional Scaling (NMDS)	
Input Data Table	
Table 'Plants' contains:	
data for calculating meadow distances () imported matrix of (dis)similarities	
using this distance measure: and the actual values are:	
Bray-Curtis distance distances	
Export distances into TSV file: Browse	
NMDS Options	
NMDS solution based on 3 axes	
Optimize solution by restarting from 0 enturbations of the initial, PCO-based configuration	
Stress formula: type 2 type 1 	
Treatment of ties in distances: primary secondary 	
Project plant species as supplementary data	

Variation partitioning

Which part of variation is due to

(a) Environment

and which to

(b) Management

and which part is

(c) shared?

two or three groups of variables



Partitioning						
		١	ariation Partition	ing Result	s for T	wo Groups
Variation Explain	ed					
Fraction	Variation(adj)	% of Explained	% of All	DF	Mean Square
a	0.30775		50.2	14.5	2	0.20054
b	0.26405		43.1	12.5	4	0.13812
с	0.041408		6.8	2.0		
Total Explained	0.61321		100.0	29.0	6	0.18126
All Variation	2.1153			100.0	19	
Copy Significance Test	ts					
Tested Fraction	FF	,				
a+b+c	2.3 0.	001				
a	2.5 0.	.004				
b	1.7 0.	.002				
Сору						
Group Members						
First Group A1	. horizont	•				
Second Group Ma	anagement	•]			
Copy Members						

- ---- -

Compared groups are represented by circles in the following diagram. Lower case letters label individual estimated fractions and are referred in the summary tables above



Principal response curves (PRC)

Method to show main effect + interaction "How does the effect of a pesticide change over time?



The later and a stand second sec

Principal response curves (PRC)(2)

Specify Time and Treatment factors

Analysis Setup Wizard: Principal Response Curves (PRC)



Principal response curves test and display treatment effects that change across time. Specify here the two factors coding temporal and treatment effects, respectively. Further, you should specify quantitative time values corresponding to individual levels of the temporal factor.

Temporal Factor	Treatment Factor
Dose	Dose
Week	Week

Specify time values for horizontal axis (default often good)

Temporal Factor Levels Translation

Specify numeric time value for each factor level:

Level	name	Tir	ne va	
Wk-4		-4		Ξ
Wk-1		-1		
Wk0.1		0.1		
Wk1		1		Ŧ
•	111		Þ	



PRC diagram: Invertebrates~ treatment.time | time



Graph 1 in Canoco5\Samples\Advanced\PRC.c5p



Canoco in faecal transplant study (1)

Development of microbiota in patients: PCA





Feces transplant study: Fuentes et al 214 ISME J. Data: **microbiota taxa** (Susana Fuentes, W. de Vos)

Canoco in faecal transplant study (2)

Development of microbiota in patients



Feces transplant study: Fuentes et al 214 ISME J. Data: **microbiota taxa** (Susana Fuentes, W. de Vos)



Co-correspondence analysis

How are two compositional data tables related?

e.g.

plant and beetle communities

(Schaffers et al. 2008)

auto- and heterotrophic microbial assemblages

(Alric et al. 2018, Mol Ecol Res)

.



Co-C	orresponde	ence Analy	sis	Summa	ary	Graph 2					
			Co-C	Corresp	onde	nce Analy	vsis (CoCA) Res	ults	
Shared	l case weigh	ts are: take	en froi	m first	table						
Fotal ii	nertias:										
Rootla	counts	3 0883									
Diant		5.5005									
Pidrit	abundances	5.7573									
Cross-	correlation b	etween CoC	A axe	s:							
1	2	3	4								
+0.95	581 +0.9414	+0.8771	+0.94	495							
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Trait-based analyses and phylogenetic relations

- Trait averages
- Functional diversity
- Doubleconstrained corresponden ce analysis (dc-CA)
- Phylogenetic corrections

AGENINGE

Species	Environment	Traits Trait Av	/erages	Fund	tional Diversity				₹
		C1	C2		C3	C4	(5	•
	Labels	Polycarpic	CNratio		seed.mass.log	SLA	height		
R1	ACHIMILL	polycarp		12.94	-0.876	19.63		2	
R2	AGROCAPI	polycarp	1	7.421	-1.444	29.54		21	
R3	ANTHODOR	polycarp	1	3.778	-0.678	29.97		14	
R4	BRIZMEDI	polycarp		33.69	-0.578	25.93		20	
R5	BROMHORD	monocarp	1	6.183	0.305	26.19		12	
R6	CAPSBURS	monocarp	Import	Dhule	gapatic Traa	X		15	
R7	CAREFLAC	polycarp	Import	Phylo	genetic free			2	
R8	CAREHIRT	polycarp	Name of	of impo	orted file:				=
R9	CARENIGR	polycarp	C:\Pro	oram i	Files\Canoco5\Sz	Browse		15	-
R10	CENTJACE	polycarp		g		brottochi			
R11	CERAARVE	polycarp	Default	brand	h length: 1.0				
R12	CERAGLOM	monocarp							
R13	CIRSARVE	polycarp	Termin	al item	is of the tree cor	respond to:		56	
R14	CYNOCRIS	polycarp	specie	s	-			1	
R15	DAUCCARO	polycarp	Itom la	hole in	the tree corresp	ond better to:			
R16	DESCCESP.C	E polycarp	a cho	rt labo	le 🦳 full Jabole	ond better to.		3	
R17	EQUIPALU	polycarp	U SIIU					36	
R18	FESTOVIN	polycarp		ОК	Cancel	Help		16	_
R19	FESTPRAT	polycarp							
R20	FESTRUBR	polycarp	2	3.679	-0.235	18.6			
R21	GALIMOLL	polycarp	1	7.856	-0.372	21.11		4	
R22	GALIULIG	polycarp	2	27.043	-0.772	29.63			

dc-CA: ter Braak et al. 2018 <u>http://rdcu.be/ETPh</u> Peng et al. 2021 <u>https://doi.org/10.1016/j.scitotenv.2020.142171</u>

Generalized linear models (GLM)

Via

- GLM template for ≥ 1 predictors
- Graph | Attribute plots
- 1 predictor:
 - Multiple response curves in single graph
- 2 predictors:
 - Contour plot



GLM Summary Lo	g Cases	ExplVars
Response variable:	Pardnigr	
Expected distribution:	Poisson	with log link function
Fitted model deviance:	245.52	with 23 residual DFs
Null model deviance:	1099.3	with 27 residual DFs
Dispersion parameter:	10.56	
Parsimony (AIC-like):	351.12	
F statistic:	20.214	(DF=4,23)
p(F):	< 0.00001	

Term	b	SE	Т	p(T)
(Intercept)	-5.58977	1.731284	-3.23	0.00371
WaterCon	3.14001	0.5211395	6.03	< 0.00001
BareSand	0.0191125	0.2406353	0.08	0.93738
FallTwig	-0.847538	0.1932076	-4.39	0.00021
Refl Lux	0.0837154	0.2033162	0.41	0.68433

Copy Help

Find out how to get a method, eg. GAM (1)

Help|Help contents (Alt-h-h) opens the help system

Type GAM in search field, press Enter, gives

For quality of life

anowin5		
Hide Locate Back Forwa	ard Print <u>O</u> ptions	S
<u>Contents</u> Index Search Favor	ites	canowin5 Canoco5 Overview
Type in the word(s) to search for:	ist Topics	Display Set them and need an assistance.
Select <u>t</u> opic: Title GAM Options dialog	Found: 13 Location canowin5	 What is Canoco5 Working with Canoco5 Support for your work Canoco5 for Canoco 4 5 users
Error fitting GAM GAM estimation failed - nega Invalid DF for a GAM predictor	canowin5 canowin5 canowin5	 How to get more memory available for Cancel

Find out how to get a method, eg. GAM (2) Look in manual or use on-line help as follows:

- Help|Help contents (Alt-h-h) opens the help system
- Type GAM in search field, press Enter
- Click GAM options dialog
- Scroll down in the help page to find

```
Getting Here: You
```

or when specifying :

where it says:

Use one of the commands in *Graph / Attribute plots* submenu (use the *Model Options* button)

Type: response curves \rightarrow topic Response curves plot \rightarrow Getting Here: use *Graph / Attribute plots / <Col-*

term> response curves



Options for ordination graphs

- In Canoco, graphs belong to an analysis
- Options for graphs can therefore be found under Analysis
- Click: Analysis | Plot creation options..

Plant species Selection Sample Selection Predictor Selection	Axes selection Horizontal Vertical Plot axes: 1 2 Flip axes: 0 Plot envelopes for
	Use pies instead of symbols for samples slices based on: values presences for plant species slices based on: values presences



Classification and groups

Click Project on the toolbar

- to create classifications, groups or series¹ of samples and species
 - for use in plotting (e.g. symbols or colours per class)
 - to plot a subset
 - etc.
- See example on next slide





¹ See <u>https://doi.org/10.6084/m9.figshare.13259534.v1</u> for more example

Ellipses and transparent colours



WAGENI For

Calibration of arrows



(Graffelman & Van Eeuwijk, 2005)

E.g. PCA on

Environment data of Dune Meadows

Arrow for Moisture calibrated





Management automatic expanded to dummies

Also available in Canoco 5

- Predicted and fitted response values for constrained methods, via Data | Add new table | Predict..; Alt-d-a-p
- Calibration predicted explanatory values; imputing of missing explanatory values on basis of constrained meth. via Advanced constrained template
- Diversity indices, via Data | Add new table | Statistics; Alt-d-a-s
- Functional diversity via Alt-d-f
- Indicator values of species for a grouping
- Multiple testing and FDR
- Multi-step analyses and more...



Resources/help

- Canoco 5 Tutorial under Programs
- Canoco 5 manual: ~500 pp
- Support site with Discussion list: <u>www.canoco5.com</u>
- Demo and practical



For Canoco projects of published analyses https://doi.org/10.6084/m9.figshare.13259534

Canoco 5

💪 Canoco 5

WinTwins

🏃 Canoco5 Tutorial

Thank you!



