The characterization of volatile matter in charcoal and its implications for soil fertility

MS Defense, Tropical Plant and Soil Sciences
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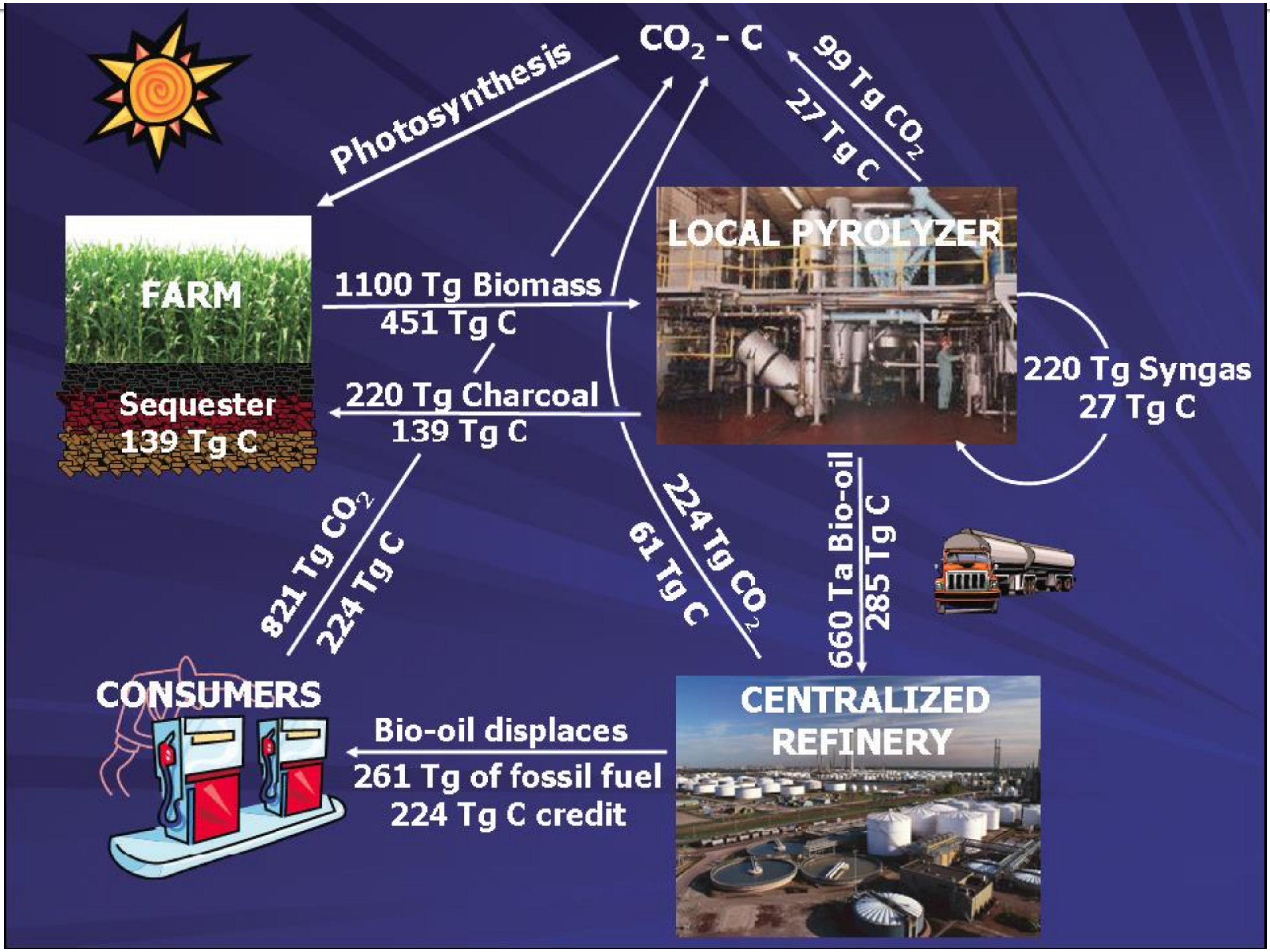
Terra Preta do Indio

Photo source: University of Bayreuth
Terra Preta Soil

Typical Upland
Amazonian Soil

"Terra Preta Novo" : Charcoal Vision



Laird, D. 2008. The Charcoal Vision: A win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. Agronomy Journal. 100:178-181.

Plant response to charcoal additions

- With no charcoal additions
- With 20% (w/w) charcoal addition

Differential effect on plant growth

Study	Charcoal feedstock	Production Temp	Crop	Rate	Positive effect on biomass	Negative effect on biomass
Lehmann et al., 2002	secondary forest	?	Cowpea	10%	almost 50% increase	
Effects of charcoal production temperature						
Chan et al., 2007	greenwaste	450°C	Radish	2%		50% decrease
Chan et al., 2008	poultry	450°C	Radish	2%	30% increase	
Chan et al., 2009	poultry	550°C	Radish	2%	almost 50% increase	
Gundale and De Luca, 2007	wildfire	High temp	*K. Macrantha*	2%	120% increase	
Gundale and De Luca, 2007	Douglas-fir	350°C	*K. Macrantha*	2%		36% decrease
Gundale and De Luca, 2007	ponderosa pine	350°C	*K. Macrantha*	2%		25% decrease

Spectrum of Charred Materials

- **Volatile Matter (VM) content**: a measure of the susceptibility of charcoal (char) to further decompose and form carbon when heated

63% VM Content

More thermally altered →

7% VM Content

Inverse relationship to carbonization temperature

<250°C

250 - 300°C

300 - 450°C

450 - 650°C

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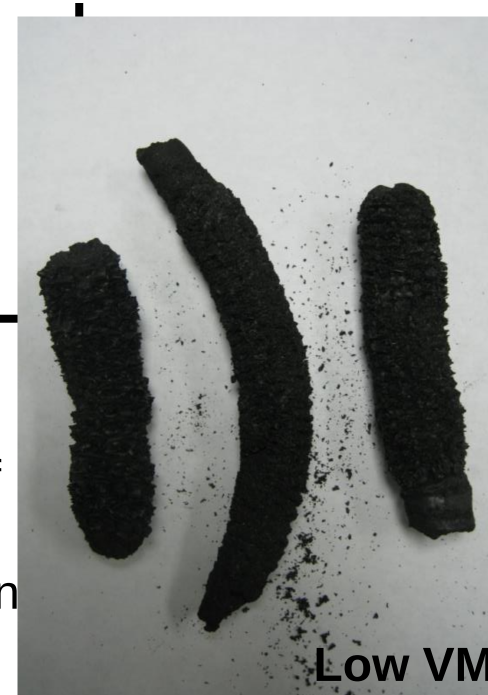
Formation of
condensed
carbon compoun



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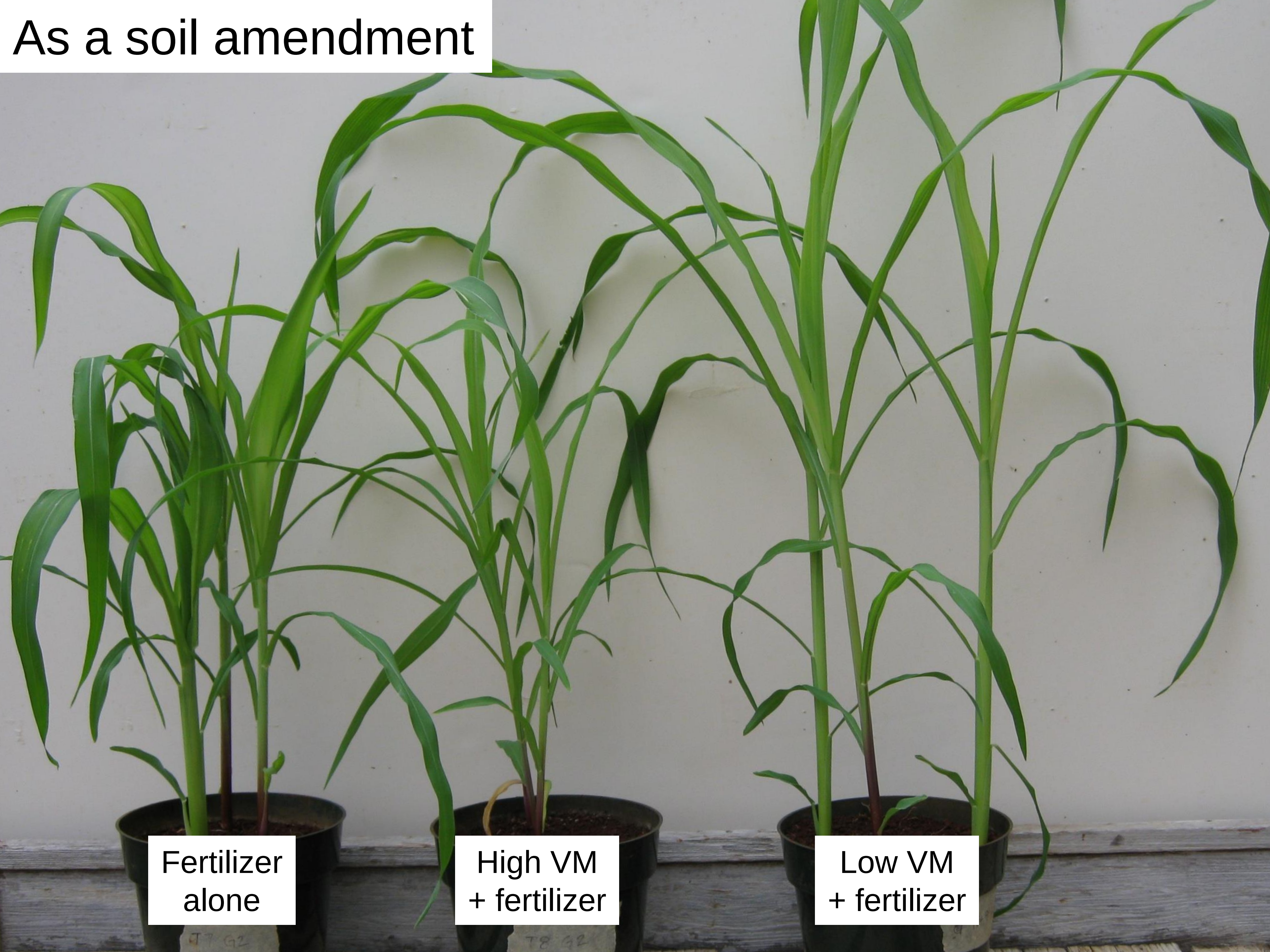
Effect of VM content on plant growth

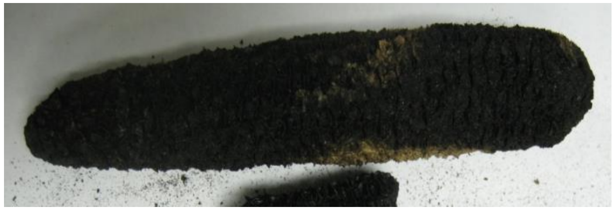
Study	Charcoal feedstock	Production Temp	Crop	Rate	Positive effect on biomass	Negative effect on biomass
Rondon et al., 2007	Eucalyptus	350°C, 33% VM	Common bean non-N fixer	10%		15% decrease
Rondon et al., 2007	Eucalyptus	350°C, 33% VM	Common bean-N fixer	10%	no effect	no effect
Deenik et al., 2010	macadamia nutshell	22.5% VM	Lettuce	5%	no effect	no effect
Deenik et al., 2010	macadamia nutshell	22.5% VM	Lettuce	10%		20% decrease
Deenik et al., 2010	macadamia nutshell	22.5% VM	Lettuce	20%		70% decrease
					Nitrogen	deficiencies
Deenik et al., 2010	macadamia nutshell	22.5% VM	Corn	10%		50% decrease
Deenik et al., 2010	macadamia nutshell	6.3% VM	Corn	10%	15% increase	

As a soil amendment

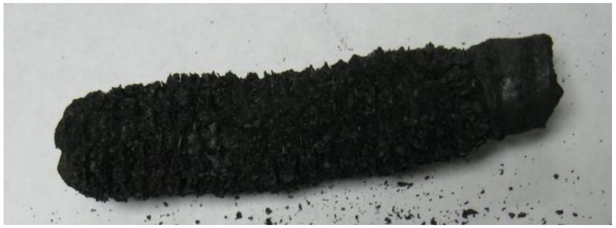


Research Goals

- To characterize the VM content in charcoal, in order to improve our understanding the effect of different charcoals on soil fertility
  - Microbial and nitrogen dynamics
  - Nutrient holding capacity



- To improve our predictions of how plants and microbes respond to soils amended with various charcoal types.



Studies

1. Charcoal characterization

2. Effect of VM content in charcoal on soil microbial and nitrogen dynamics

3. Effect of fresh and aged charcoals on soil charge characteristics (e.g. cation exchange capacity)

Study #1: Characterization

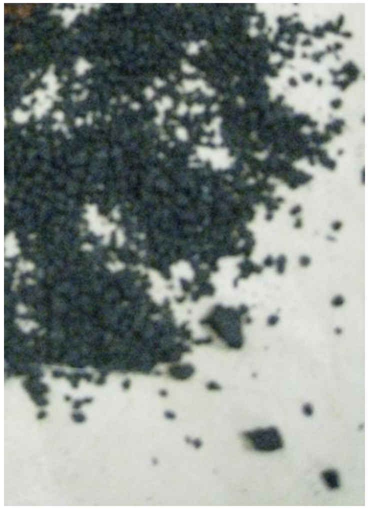
Objectives:

1. To characterize charcoals with different VM contents and feedstocks
2. To relate differences in structure and extractable molecular compounds to charcoal behavior in soil

Hypotheses:

- The VM content = phenolic compounds and hydrocarbons
- These compounds are presumably bioavailable and could potentially inhibit plant growth in the short term

Procedures for Study #1



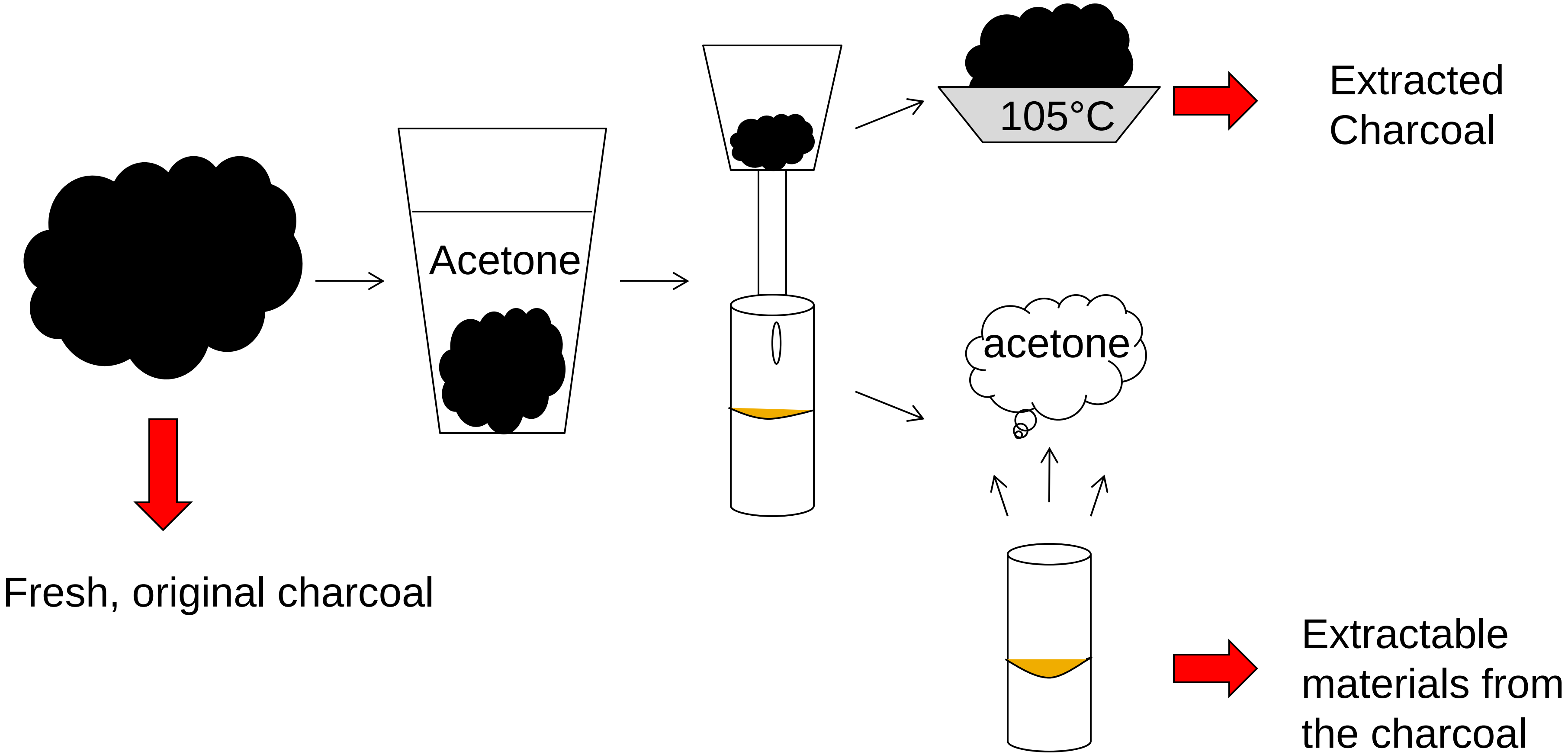
Charcoal Types

1. 63% VM corncob
2. 23% VM corncob
3. 7% VM corncob
4. 23% VM kiawe
5. Commercial activated charcoal
6. 23% VM corncob extracted with acetone

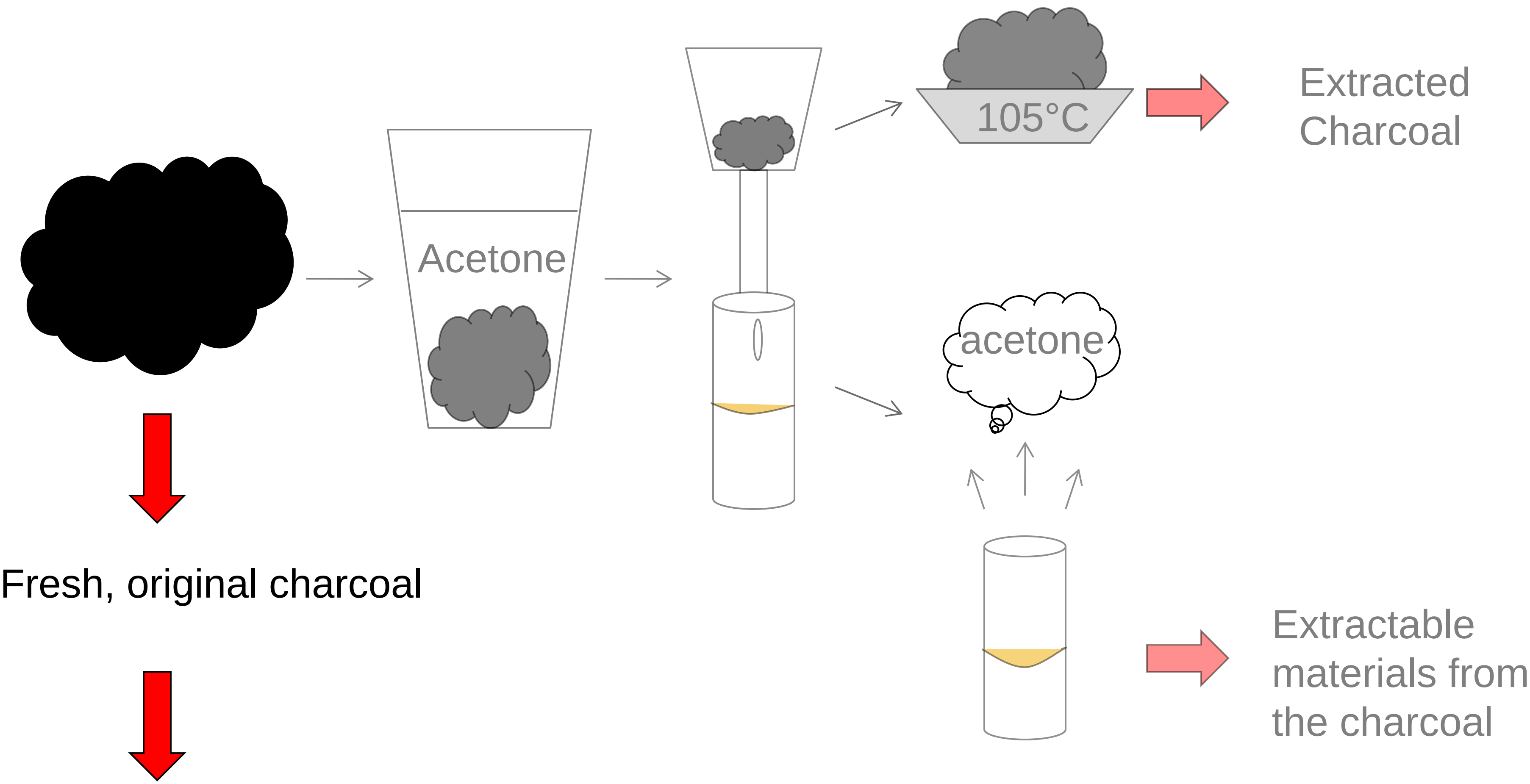
4 Techniques

1. Fourier transformed infrared spectroscopy (FTIR)
2. Nuclear magnetic resonance (NMR)
3. Gas chromatography mass spectroscopy (GC-MS)
4. Prussian Blue for Total Phenols

Fractionation of charcoal

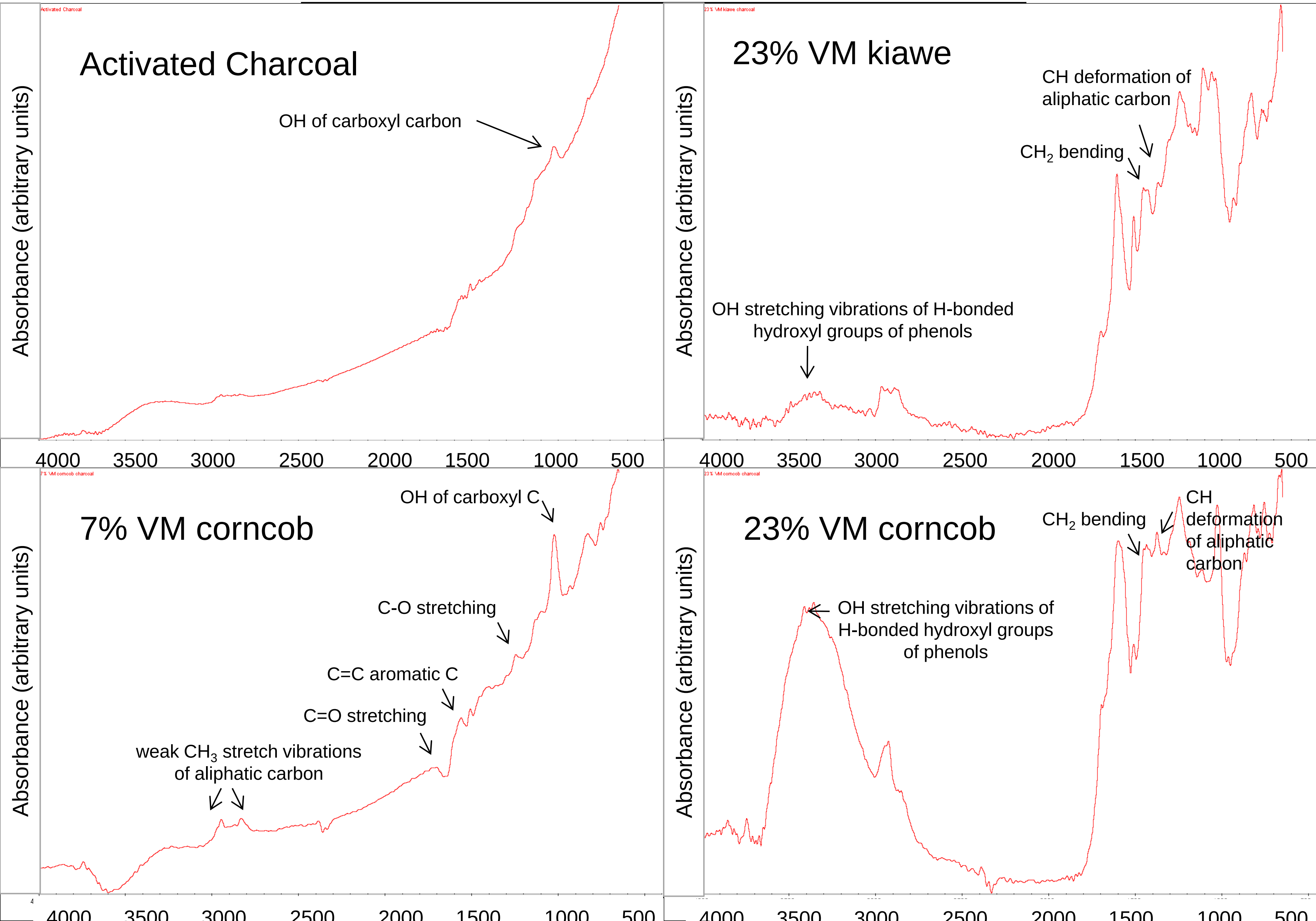


Chemical structure—NMR and FTIR

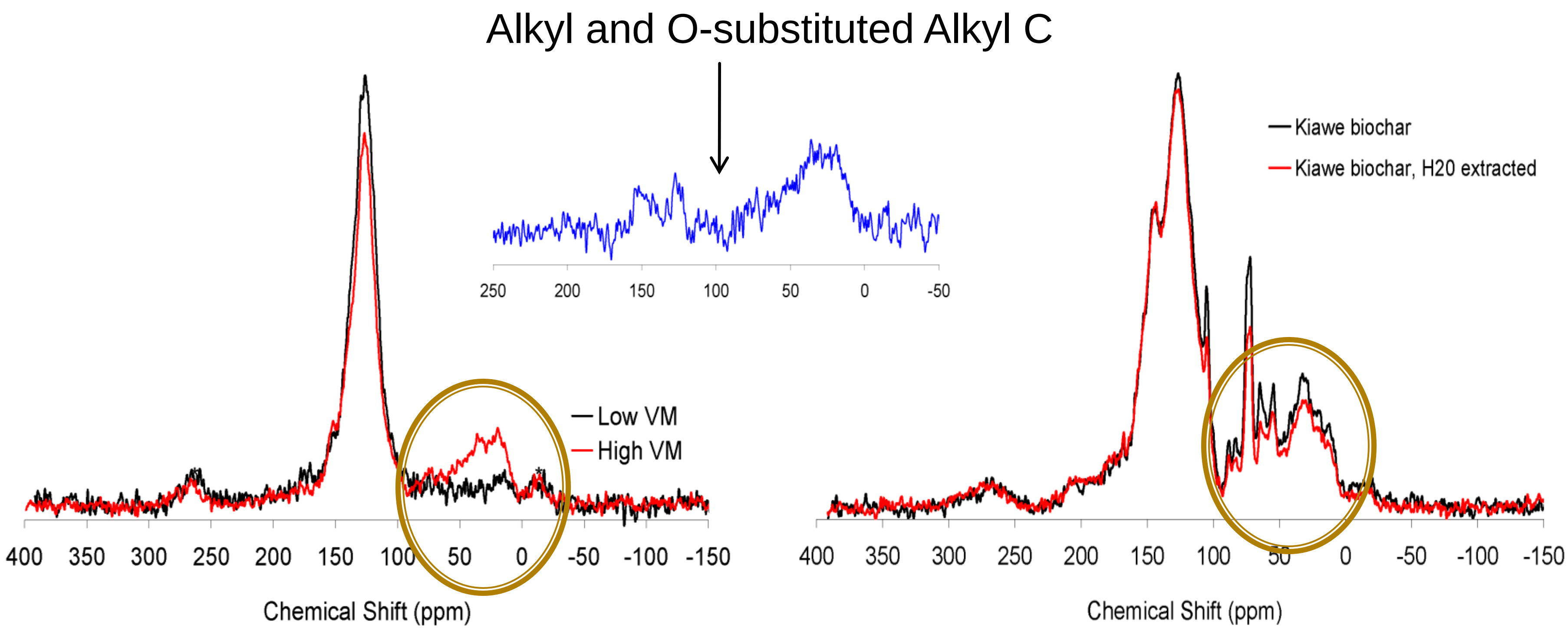


FTIR and NMR

1.FTIR—chemical structure



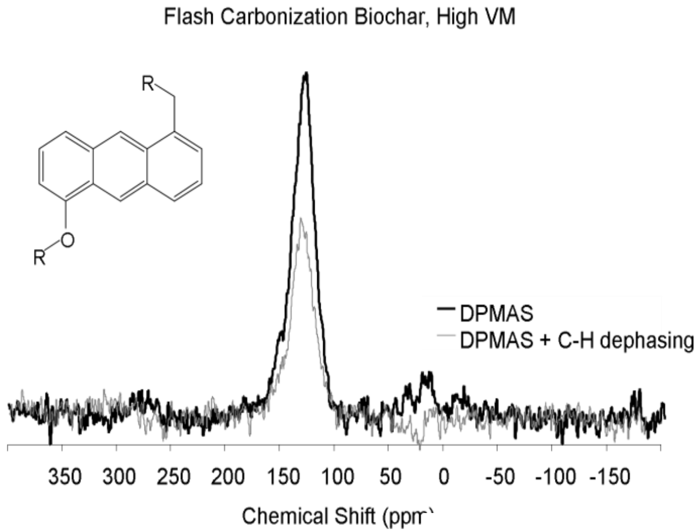
2.NMR—chemical structure

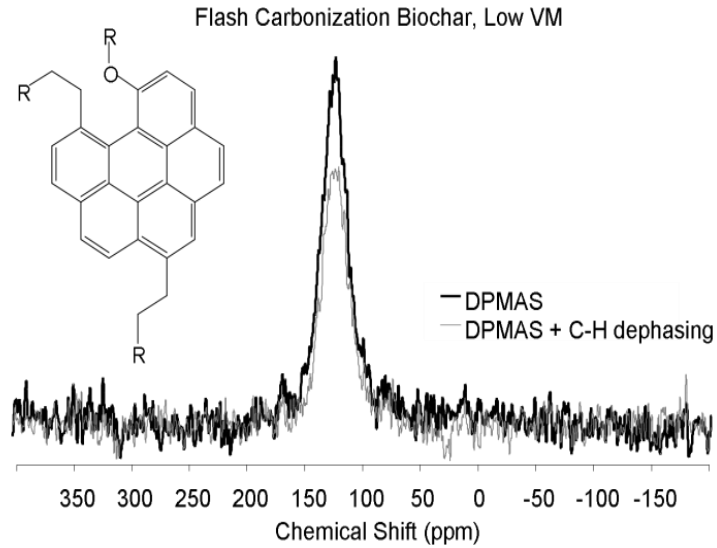


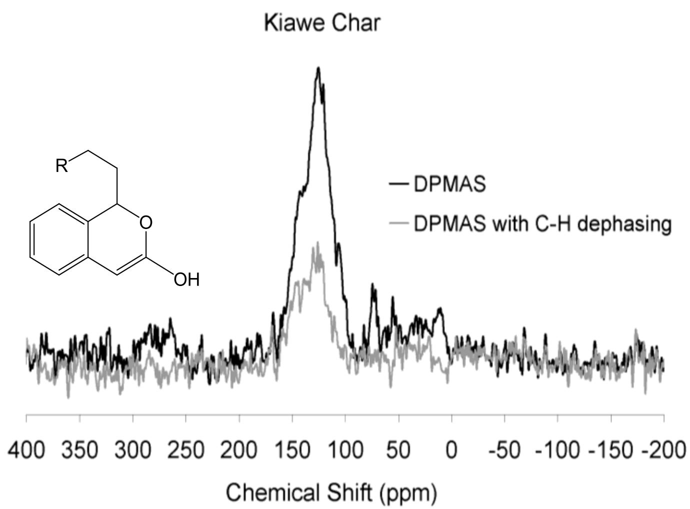
Corncob charcoals

Kiawe charcoal

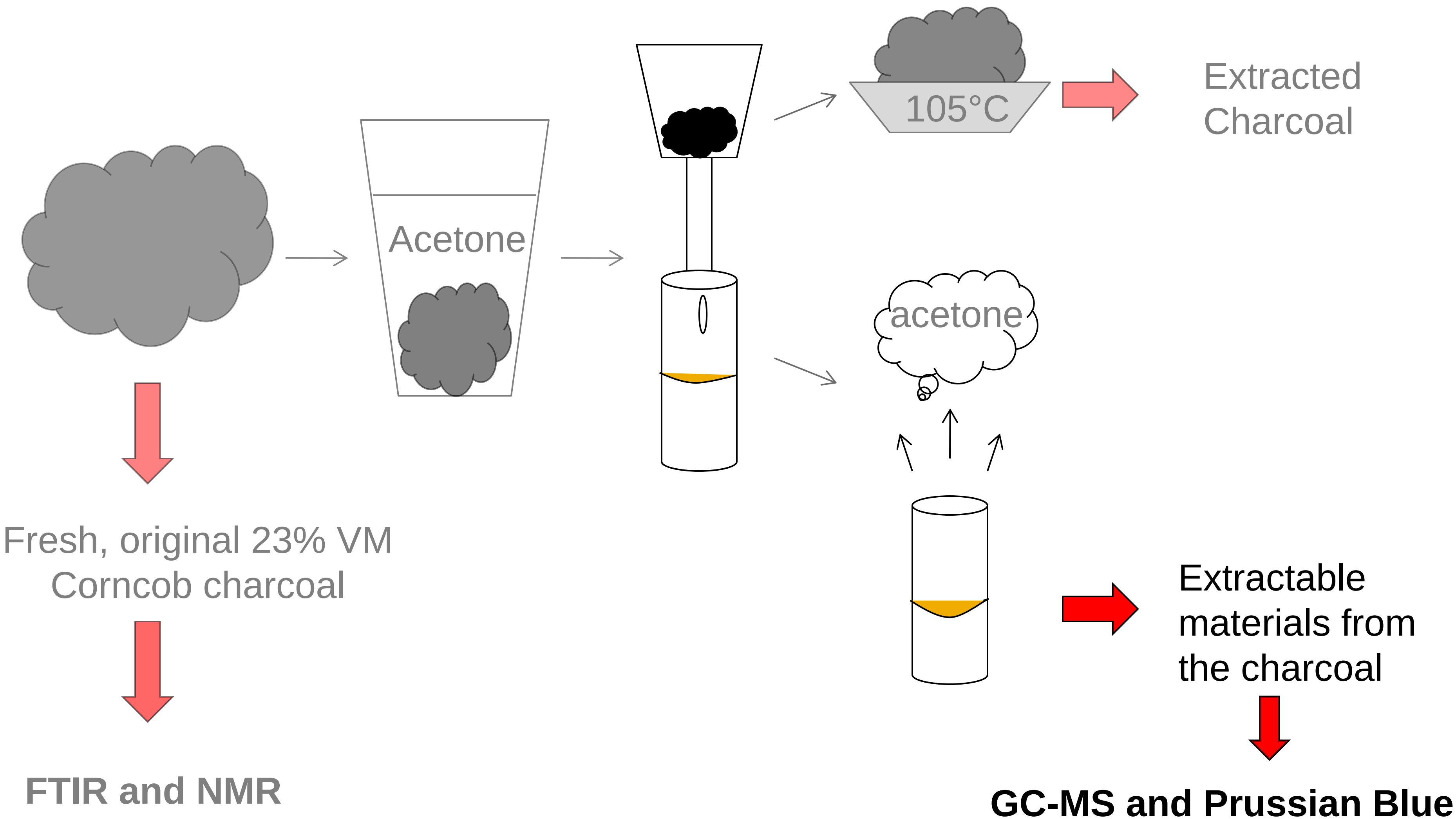
2. NMR
Hypothetical Structures



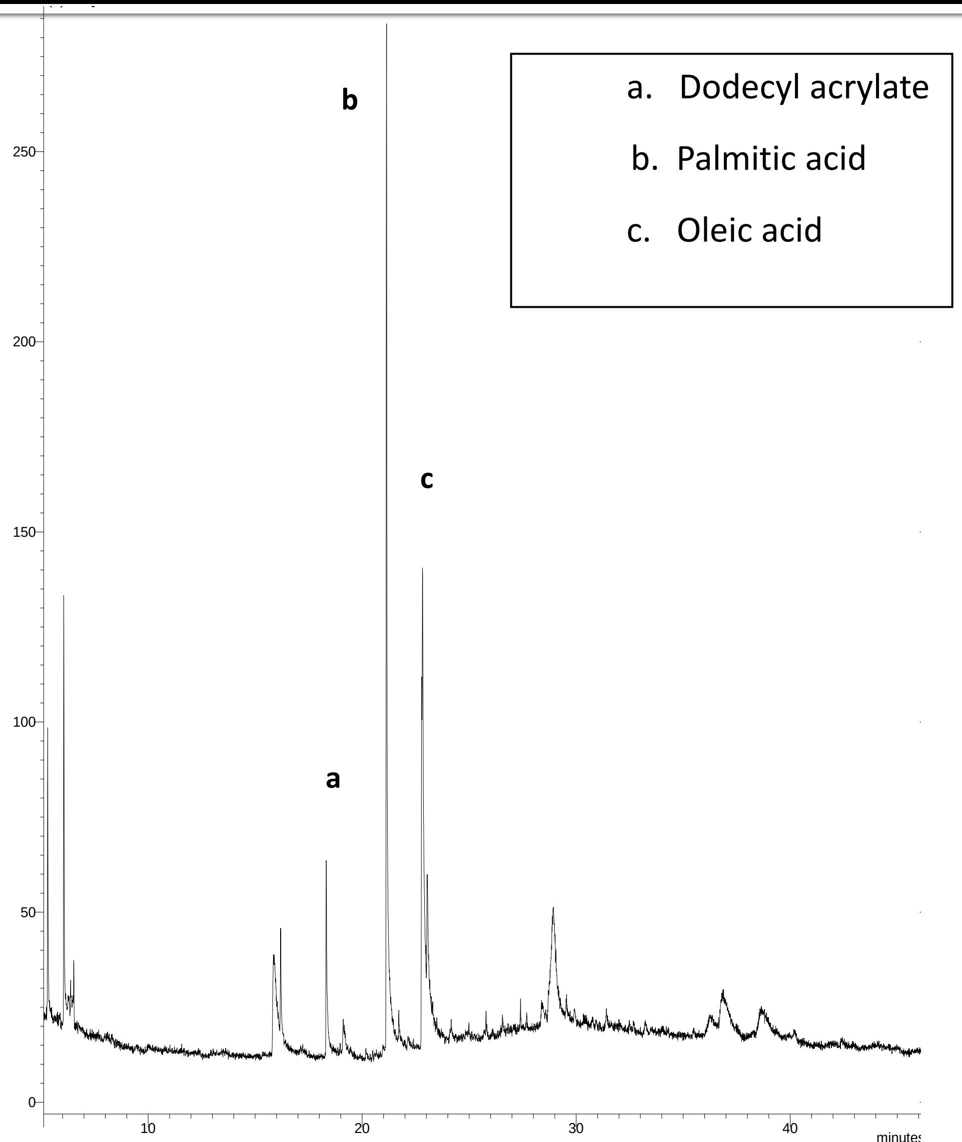




Composition of extractable components

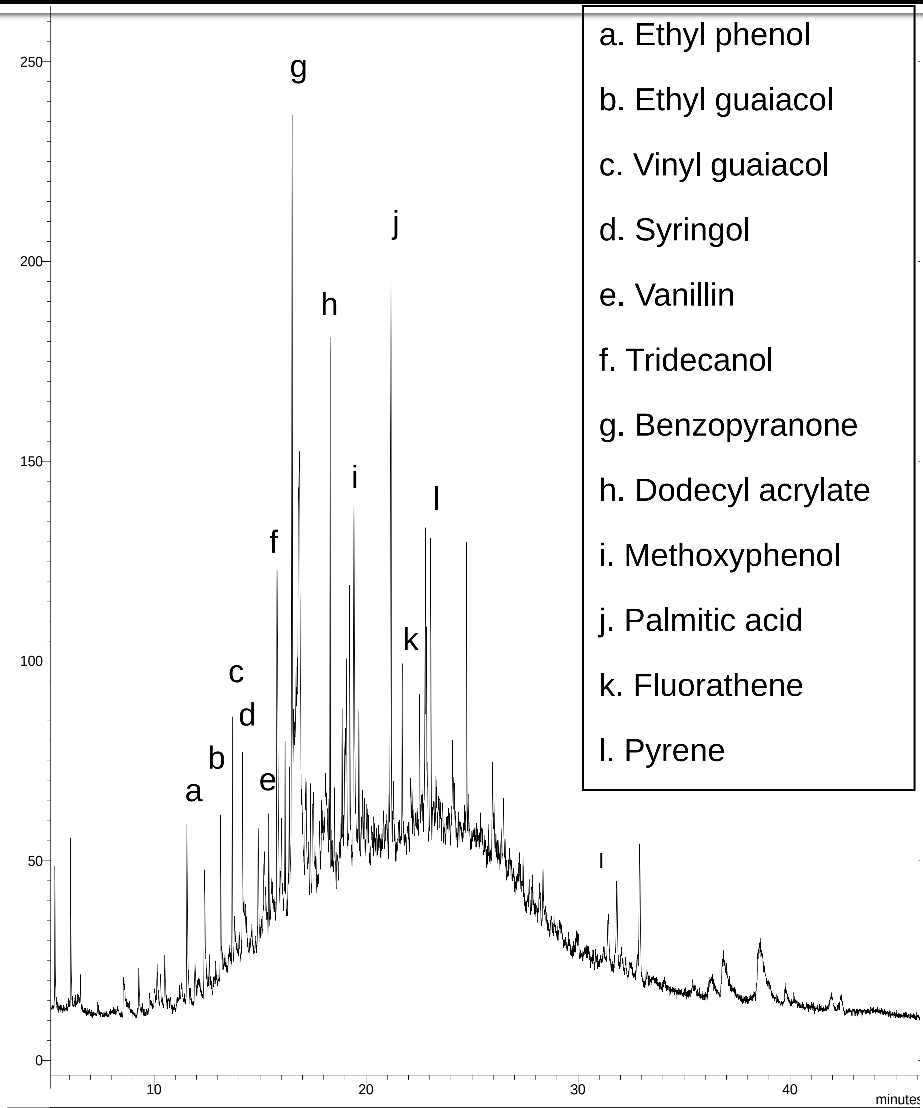


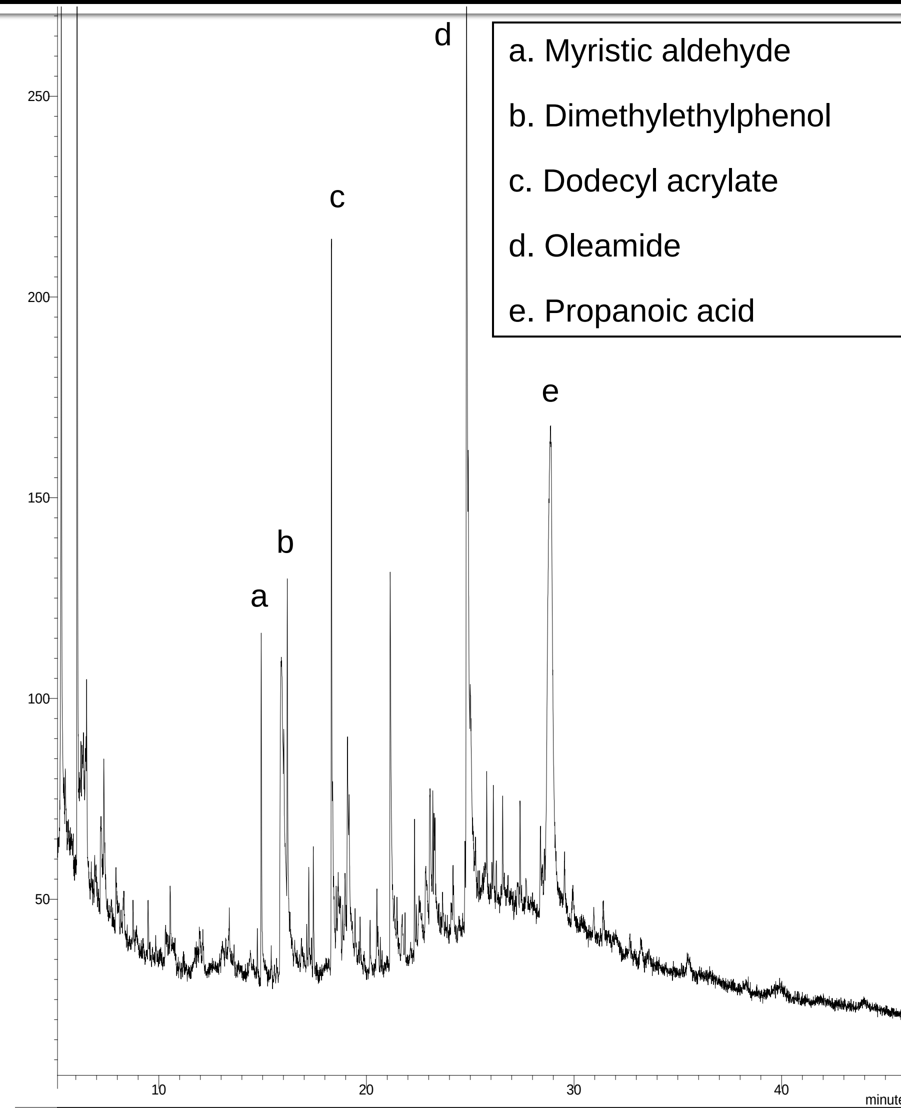
3.GC-MS—extractable compounds
Corncob feedstock



3.GC-MS

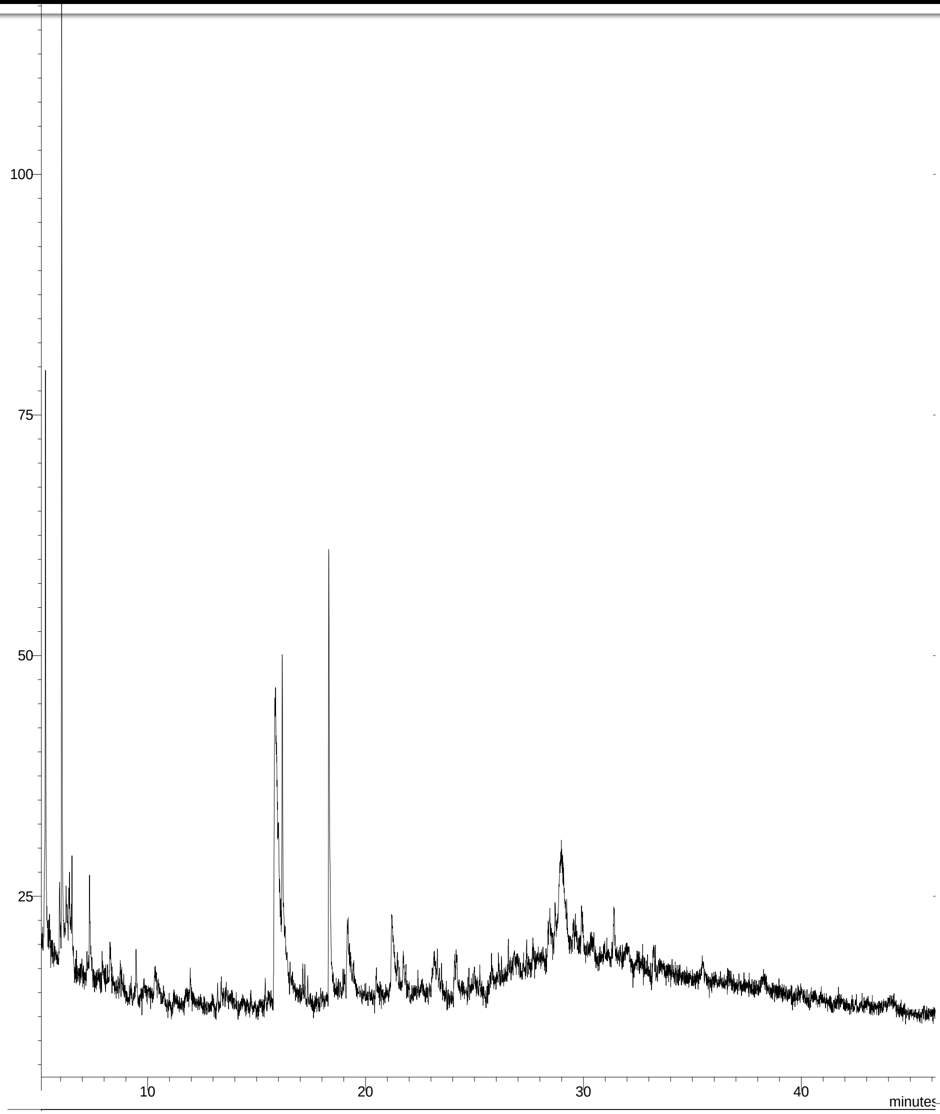
63% VM corncob charcoal   23% VM corncob charcoal



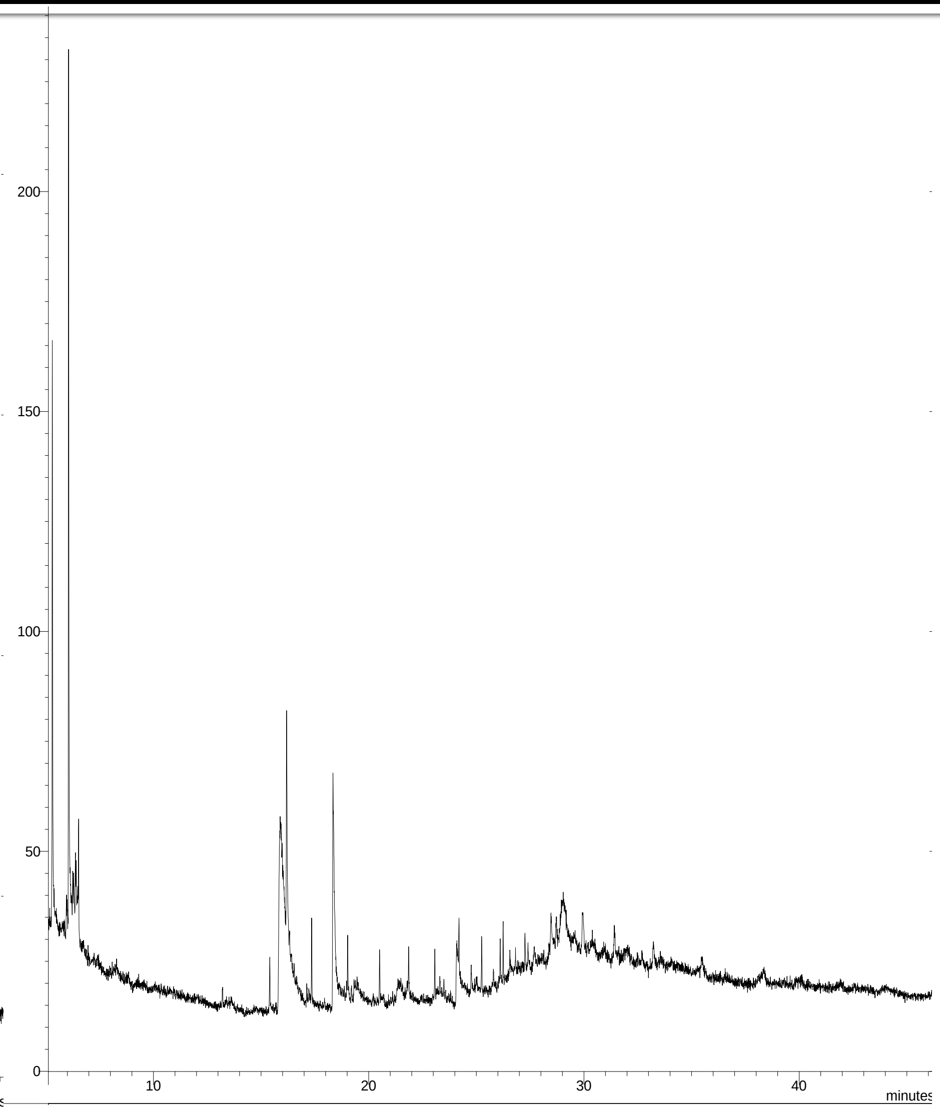


3.GC-MS

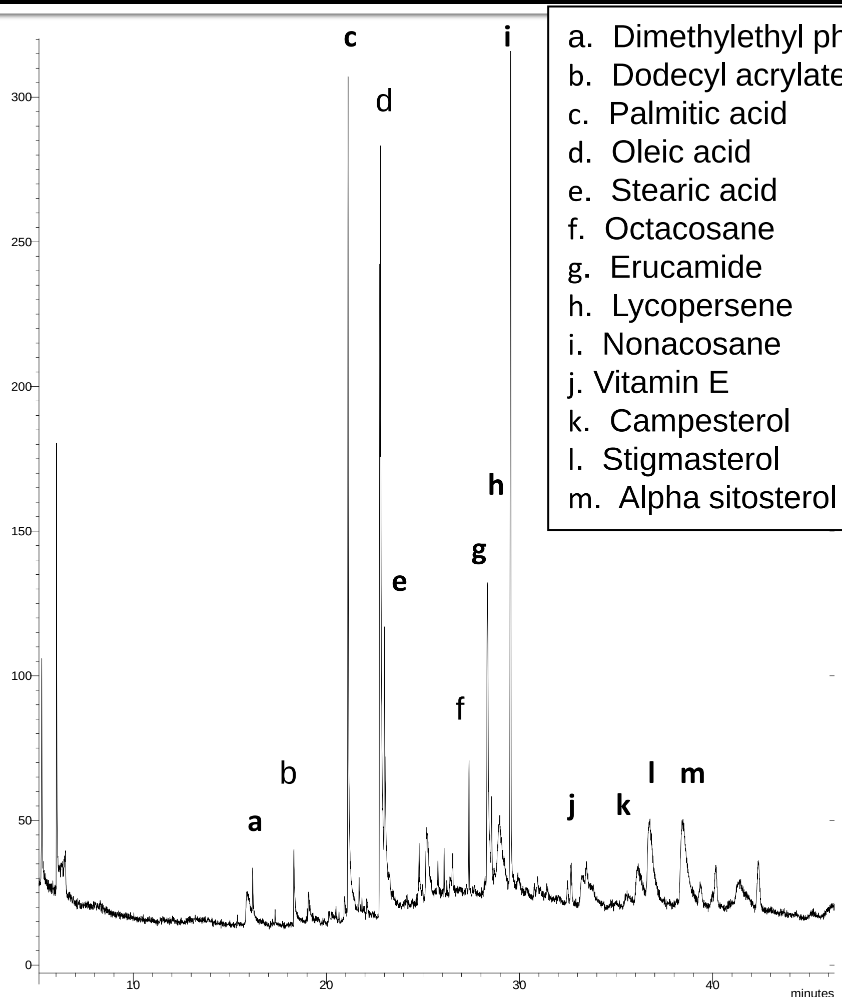
7% VM corncob charcoal



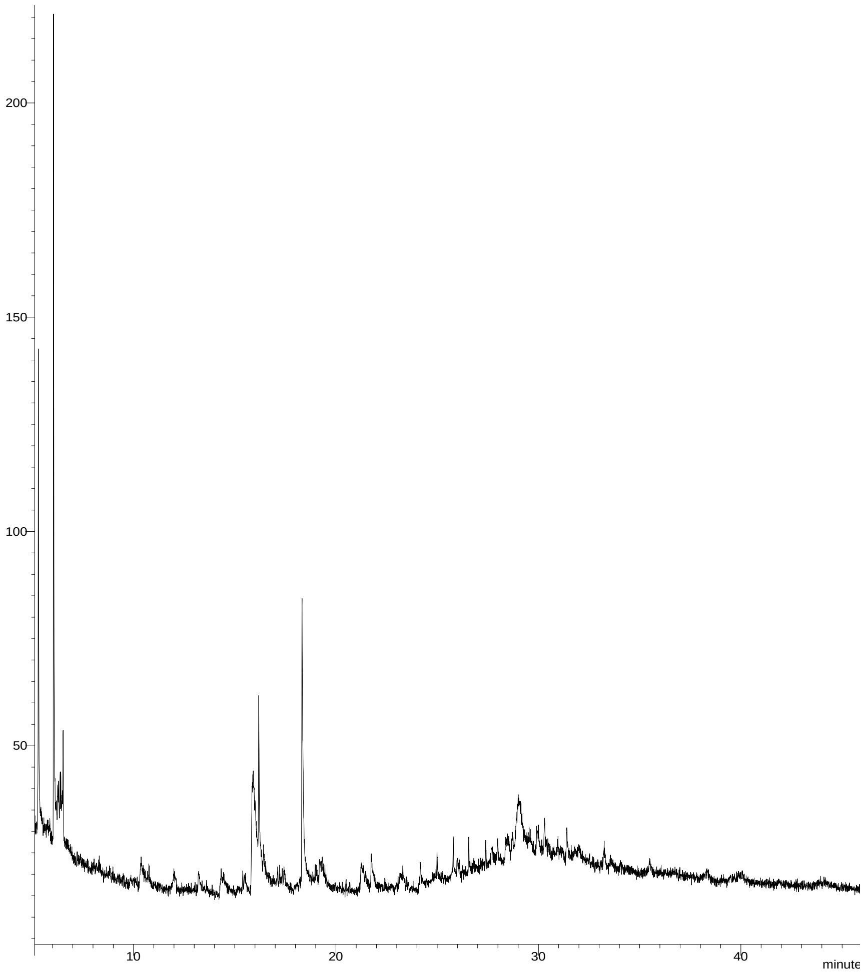
Extracted 23% VM corncob



3.GC-MS
Kiawe Feedstock and 23% VM Charcoal



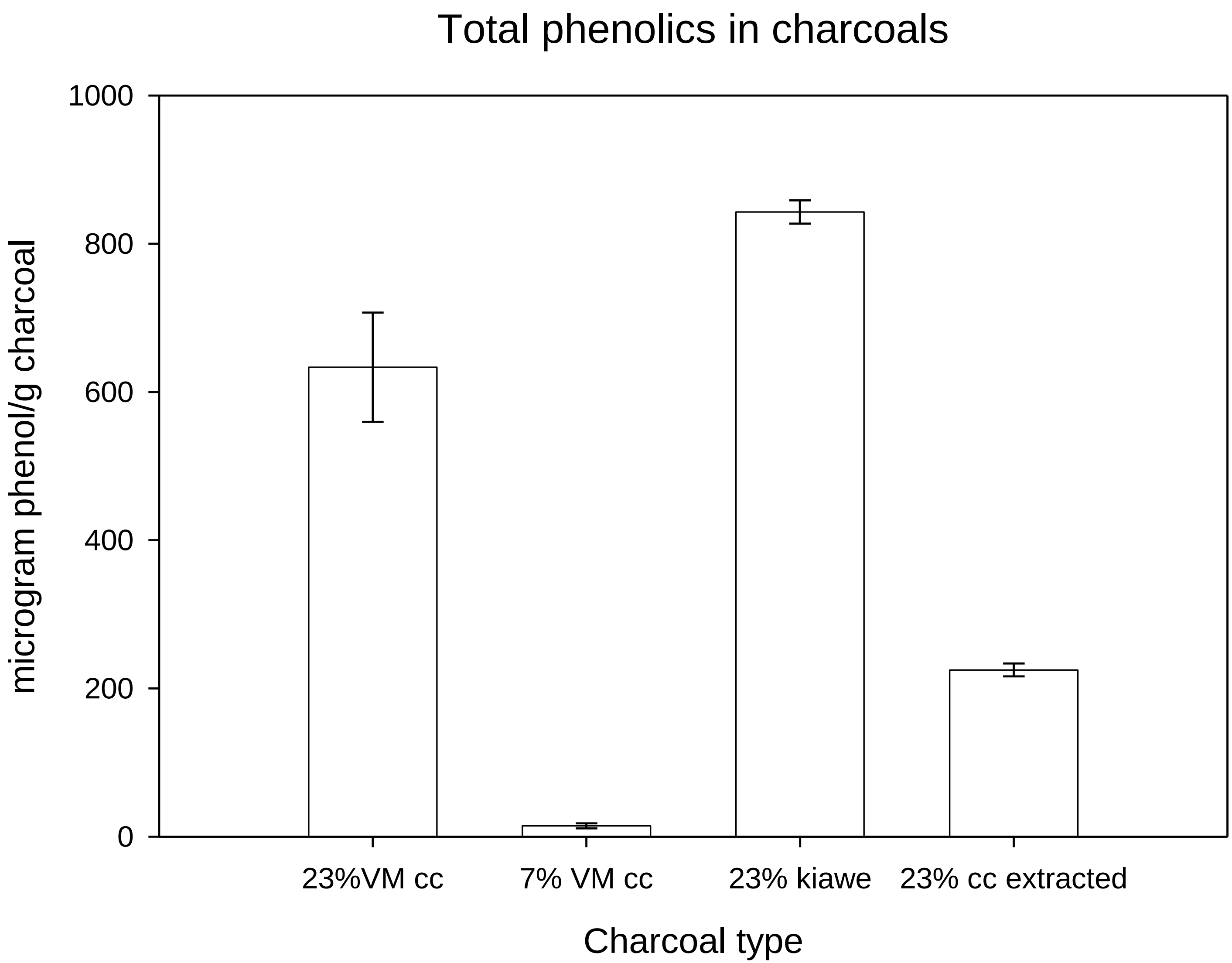
Feedstock



23% VM kiawe char

Prussian Blue for Total Phenols:

Extracted with acetone



Interpretation—Study #1

- Measurable differences in the chemical structure and composition of charcoals

VM content of charcoals = mostly alkyl carbons and oxygen-substituted alkyl carbons (NMR), as well as phenolics (FTIR)

- GC-MS able to detect a range of extractable compounds that varied among chars, particularly feedstocks. Bioavailability?

High VM corncob vs. High VM kiawe charcoal

- Unable to detect extractable compounds in kiawe char. What other types of chemical compounds makeup VM content? Py-GC-MS or HPLC?

Study #2—Effect on biological activity

Objective:

To determine the effect of VM content in charcoal on:

1. Soil microbial activity
2. Soil nitrogen dynamics

Hypotheses:

- A higher VM content will stimulate microbial growth and activity—resulting in N immobilization
- This effect will be attributed to the presence of bioavailable carbon compounds in high VM charcoal

One-month incubation—rationale

- Our previous greenhouse studies showed nitrogen deficiencies in plants amended with high VM charcoals
- High VM corncob charcoals contained hydrocarbons and an array of extractable molecular compounds
- Opportunity to determine the initial effects of VM content on microbial and nitrogen dynamics in a highly weathered subsoil

One-month incubation—procedure

Soil: Leilehua subsoil

Charcoals

1. 34% VM corncob
2. 7% VM corncob

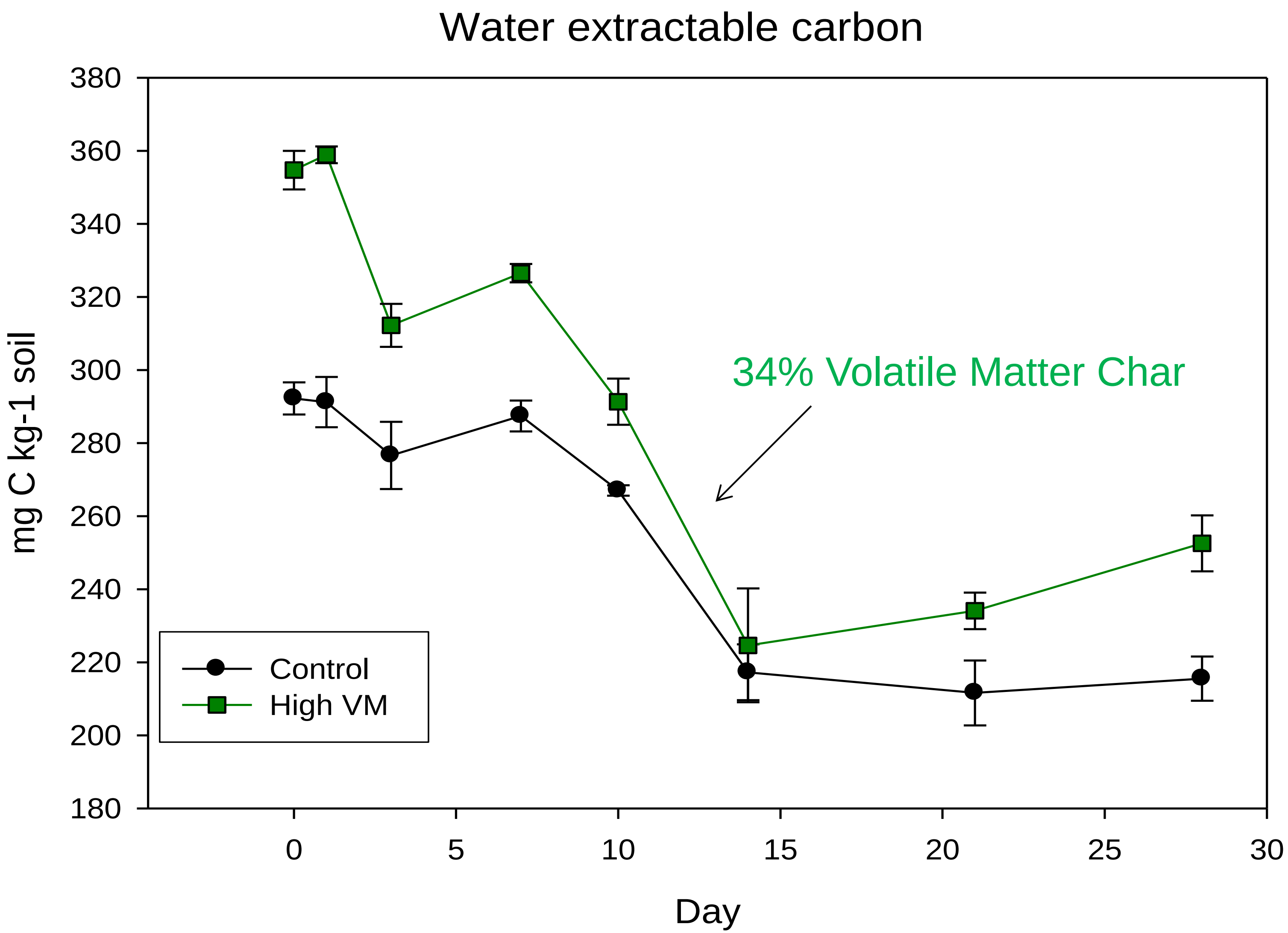
Nitrogen

1. 0 kg/ha
2. 150 kg/ha

Measurements

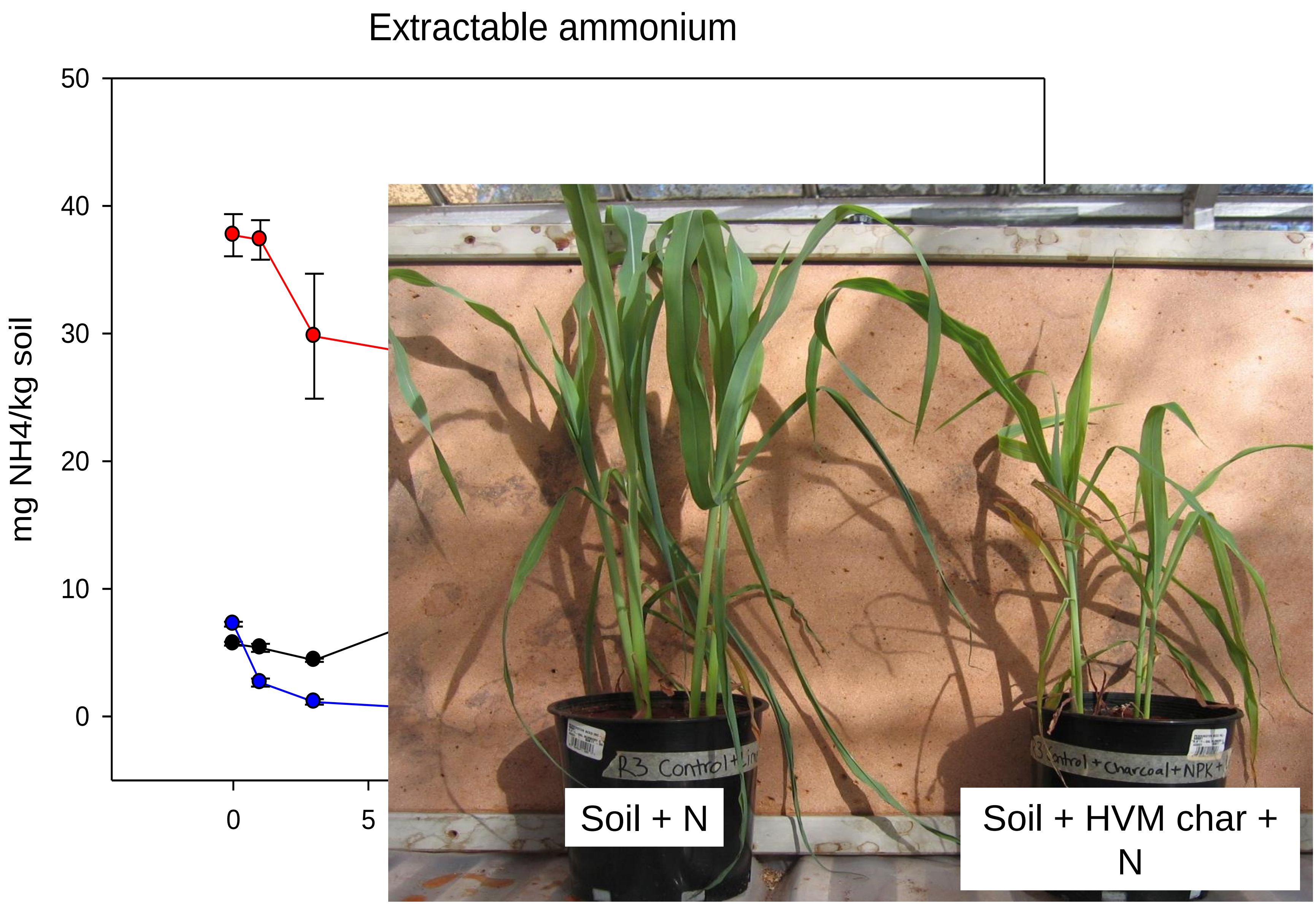
1. Water extractable organic carbon
2. Hydrolytic enzyme activity
3. Extractable ammonium and nitrate

Incubation 1: Organic carbon



Incubation 1: Biological Activity

Incubation 1: Nitrogen



Two-month incubation—rationale

- We observed the stimulation of microbial activity and nitrogen immobilization in the one-month incubation by high VM corncob charcoal, as hypothesized.

- A later greenhouse study showed that a high VM kiawe charcoal did not have a negative impact plant growth.
  - Remember GC-MS did not detect any carbon compounds

- In a longer study, we aimed to compare the effects of corncob and kiawe charcoals with equivalent VM contents on microbial activity.

- What if we leached the charcoal first with acetone? Same effect?

Two-month incubation—procedure

Soil: Leilehua subsoil

Amendments
1. Corncob feedstock
2. 7% VM corncob
3. 23% VM corncob
4. Kiawe feedstock
5. 23% VM kiawe
6. Extracted 23% VM corncob

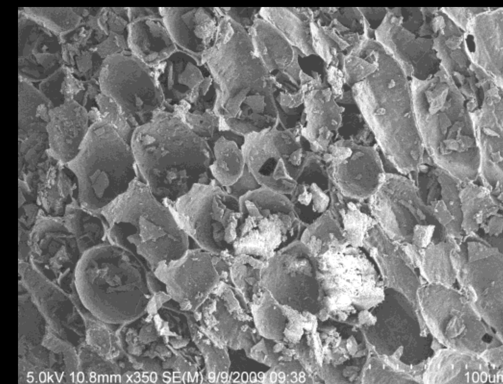
Nitrogen: none added

Measurements
1. Hydrolytic enzyme activity
2. Extractable ammonium and nitrate

SEM micrographs of materials

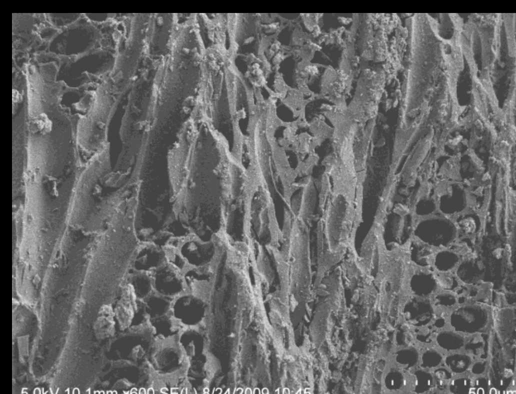
Corncob husks

23% VM corncob



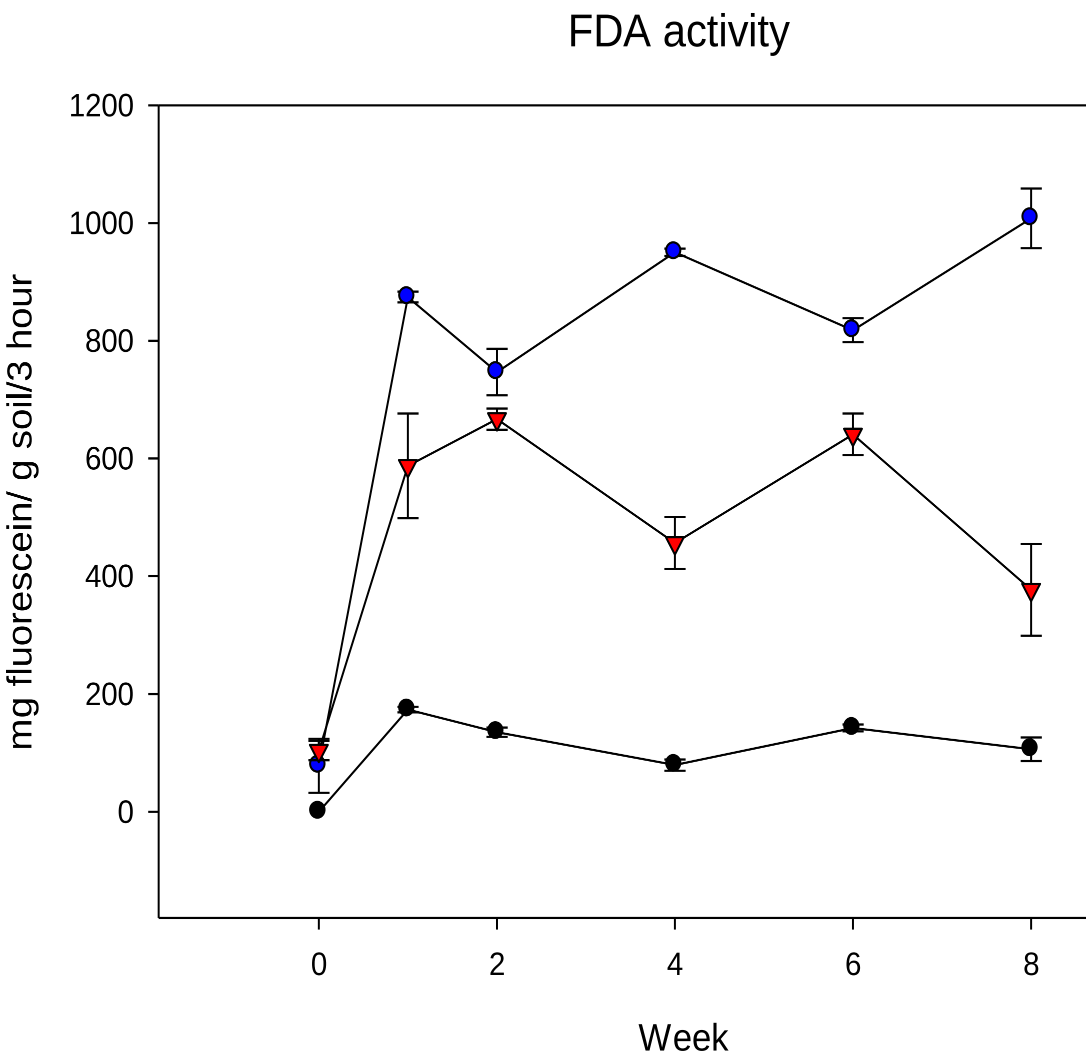
7% VM corncob

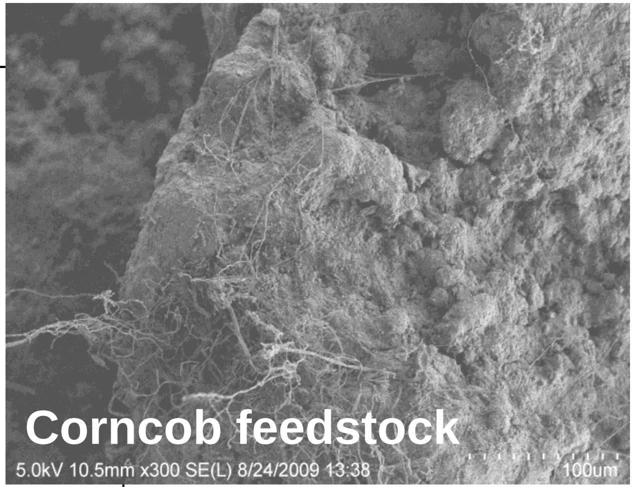
Kiawe feedstock

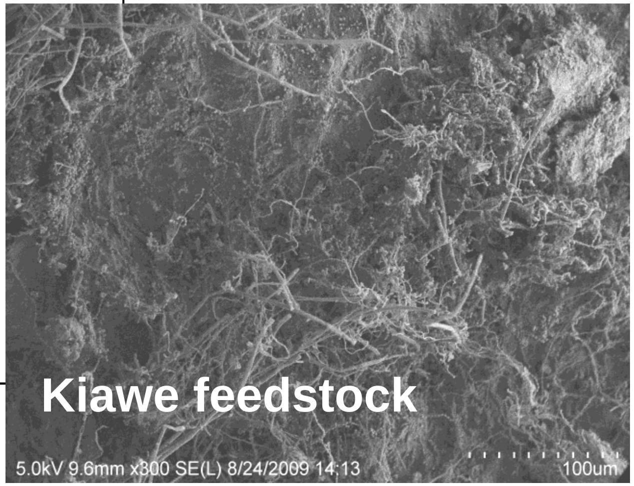


23% VM kiawe

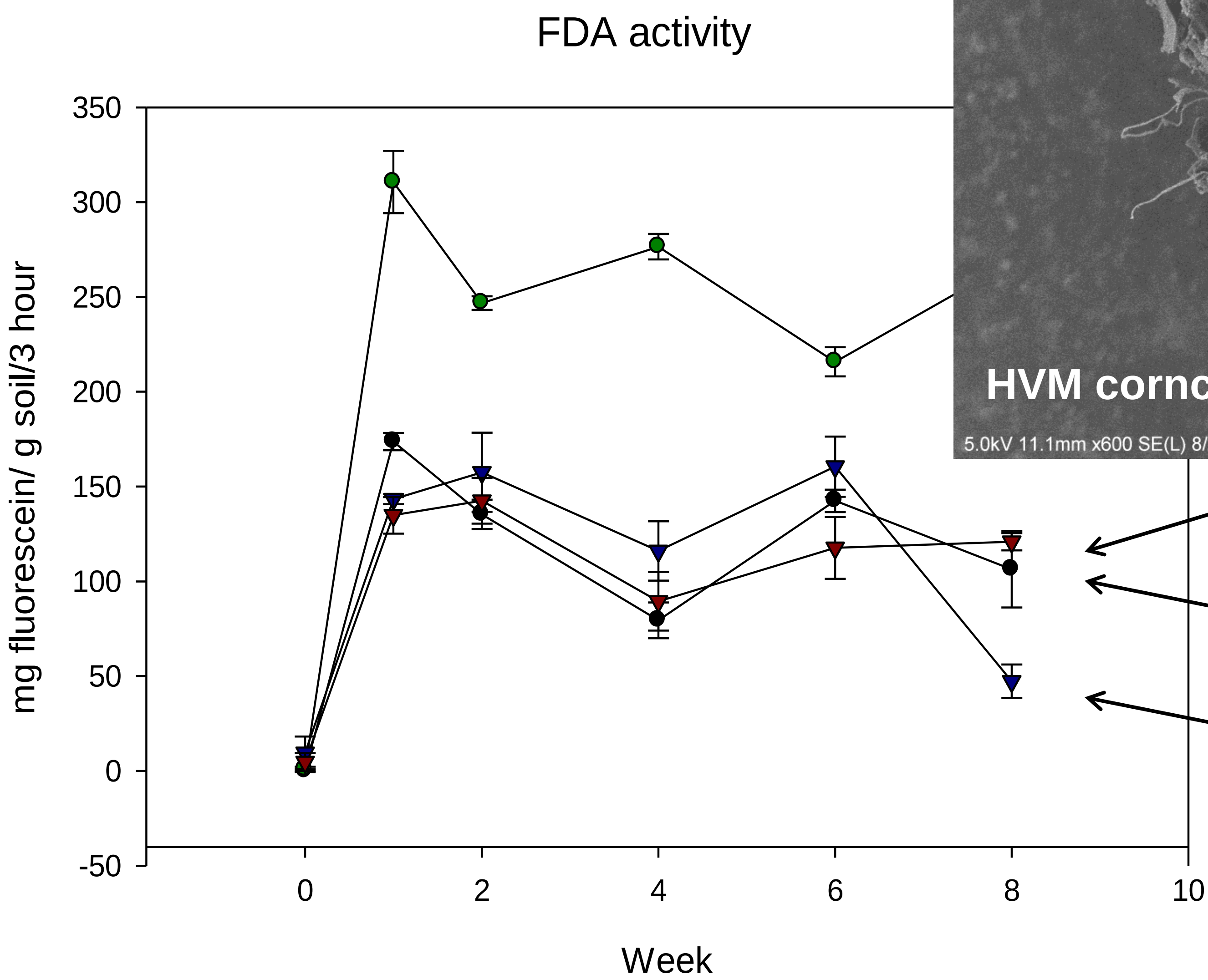
Incubation 2: Biological Activity

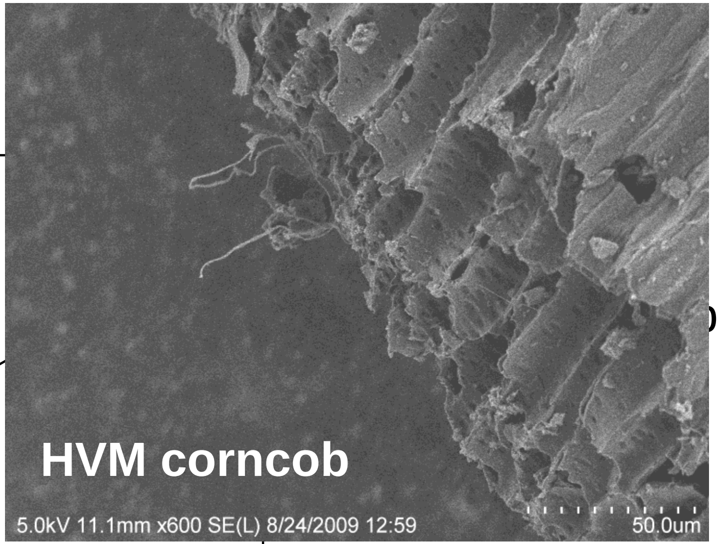






Incubation 2: Biological Activity



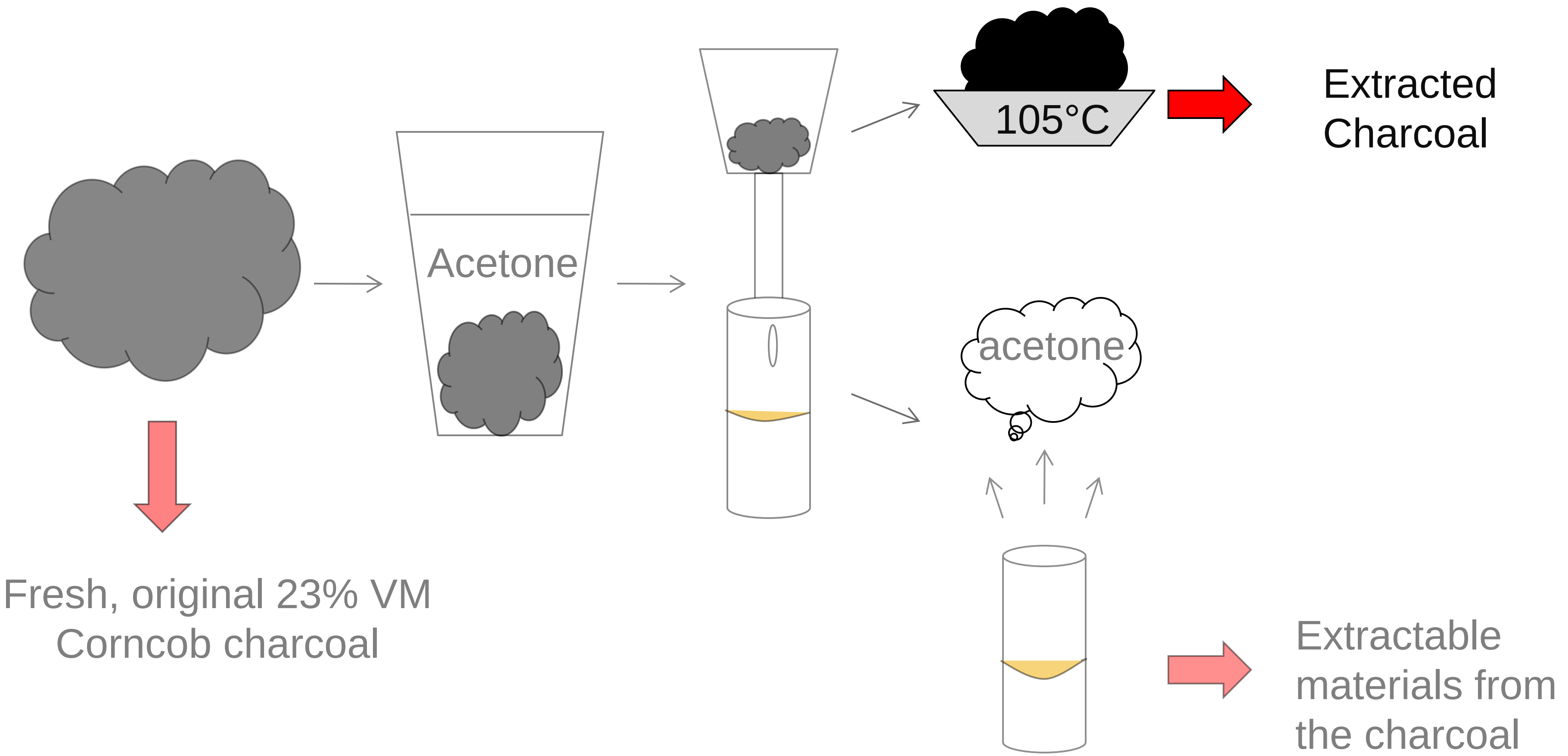


23% VM kiawe

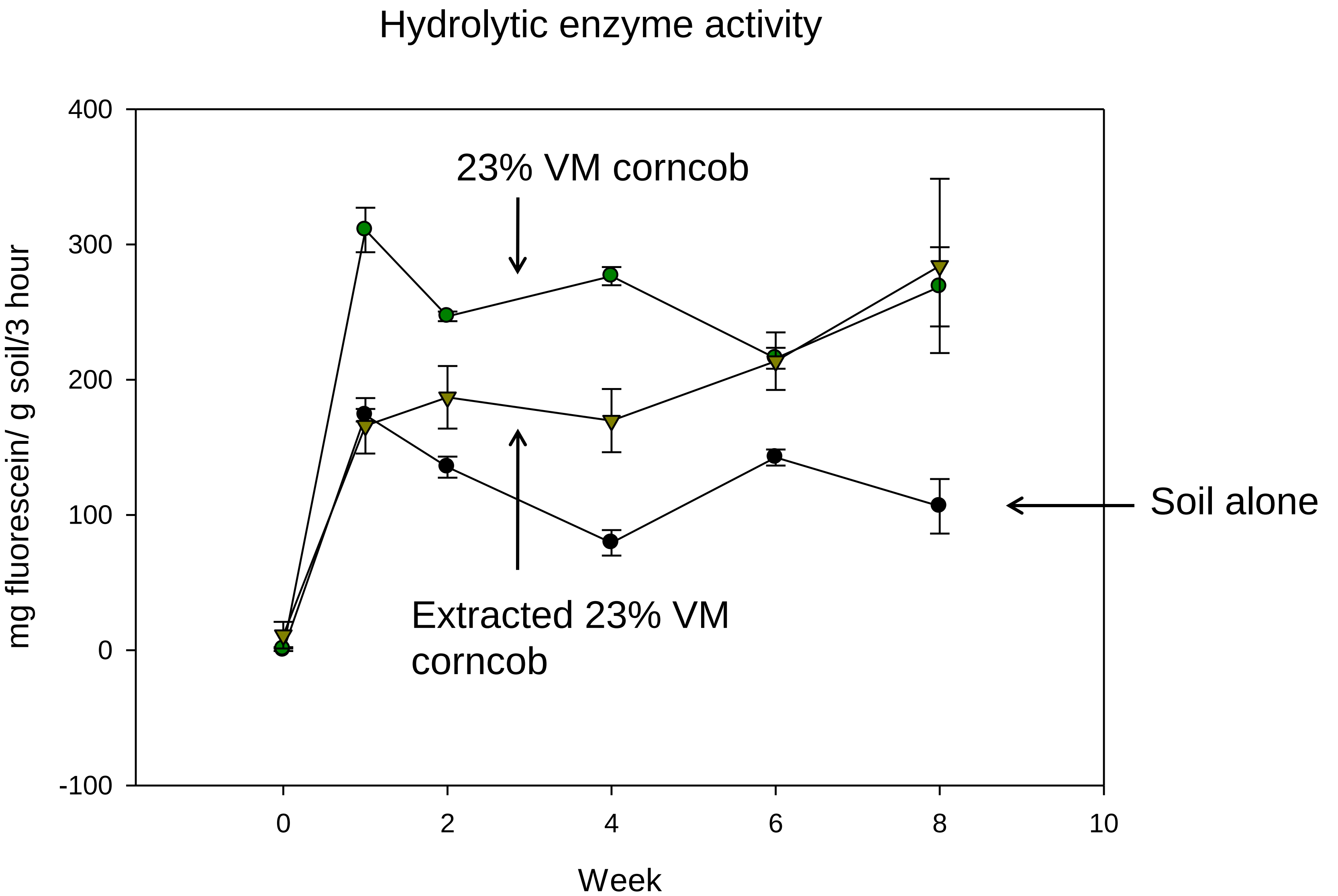
Soil alone

7% VM corncob

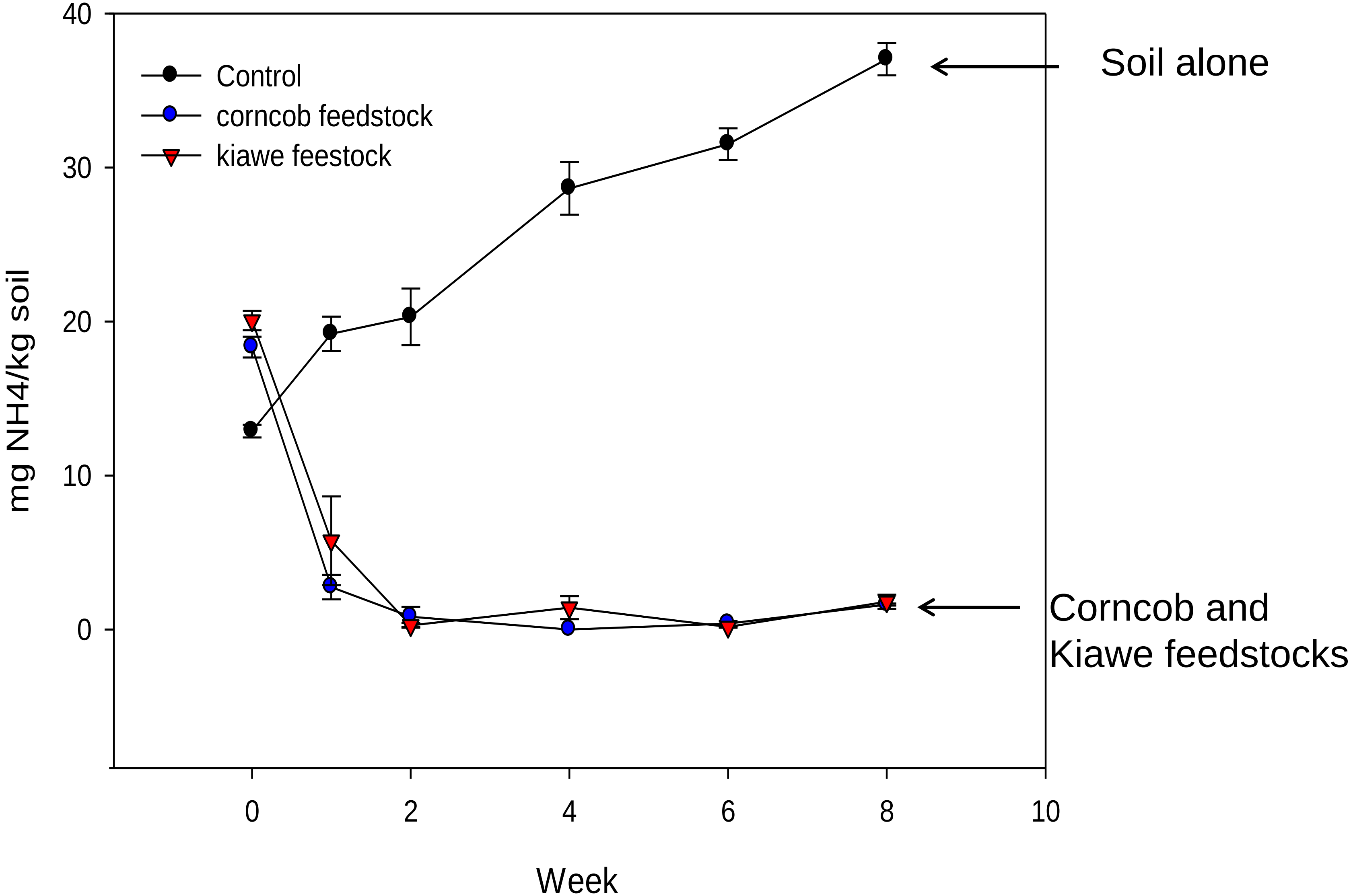
Fractions of 23% VM corncob char



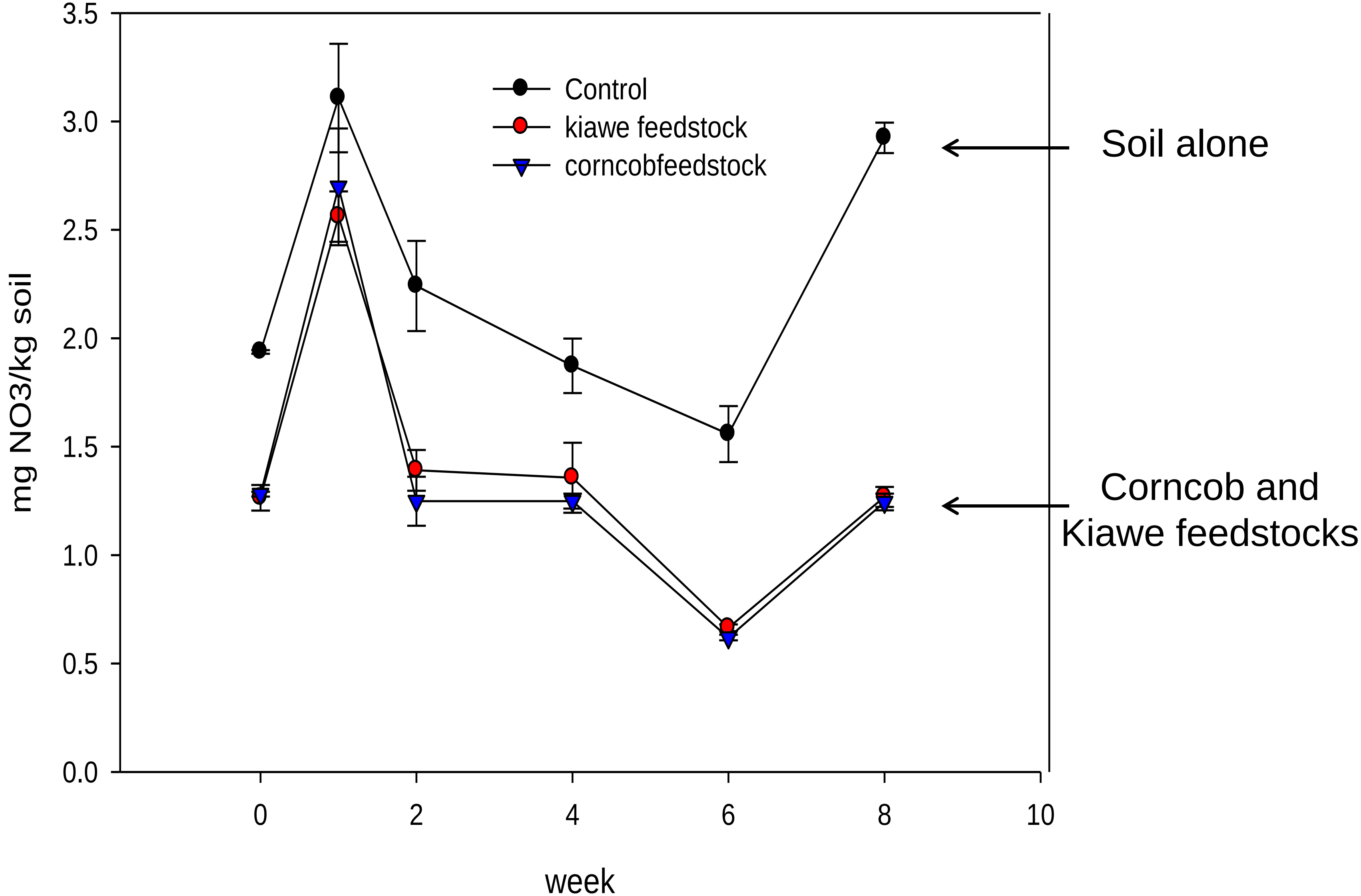
Incubation 2: Biological Activity



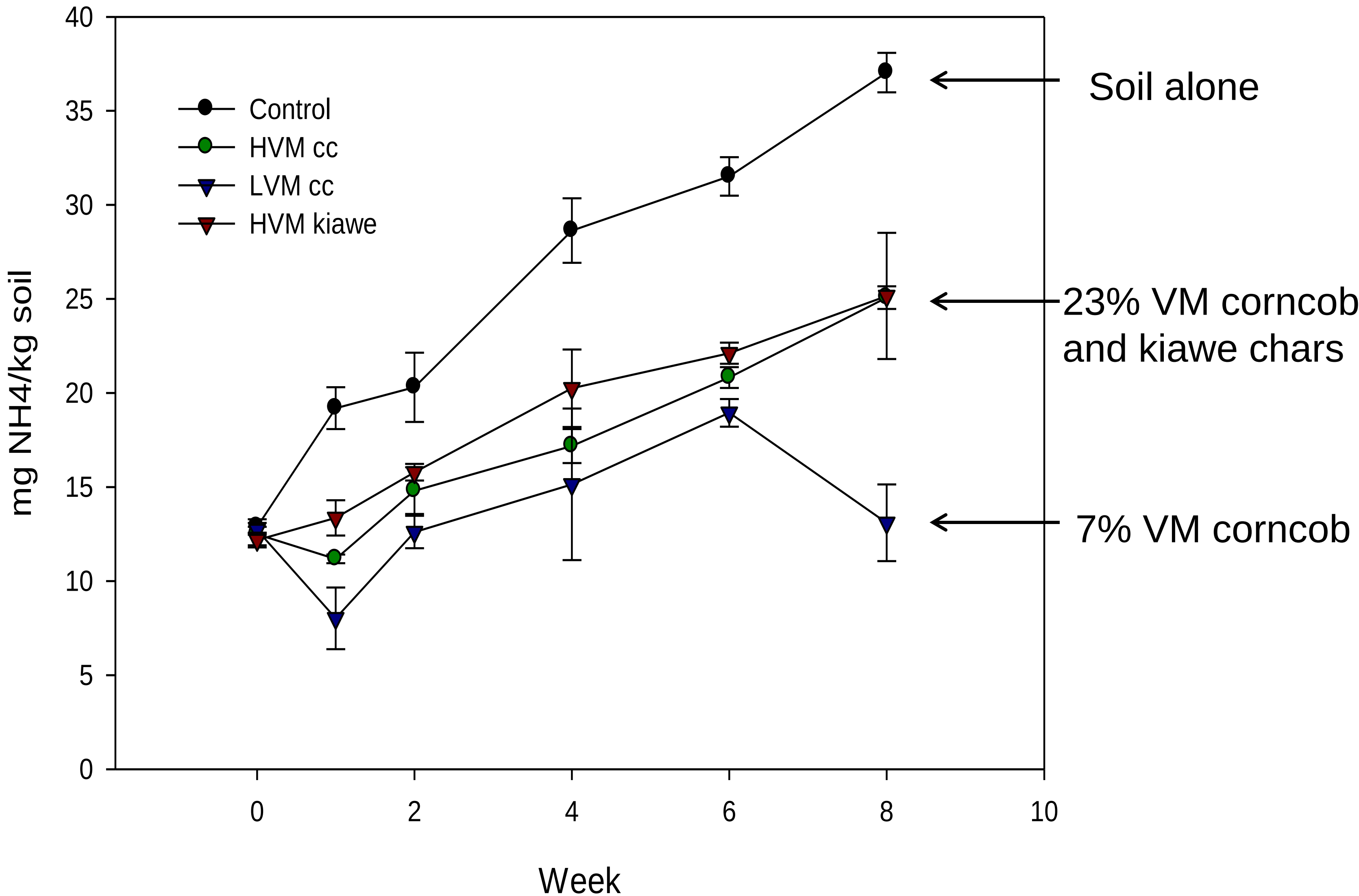
Incubation 2: Ammonium Nitrogen



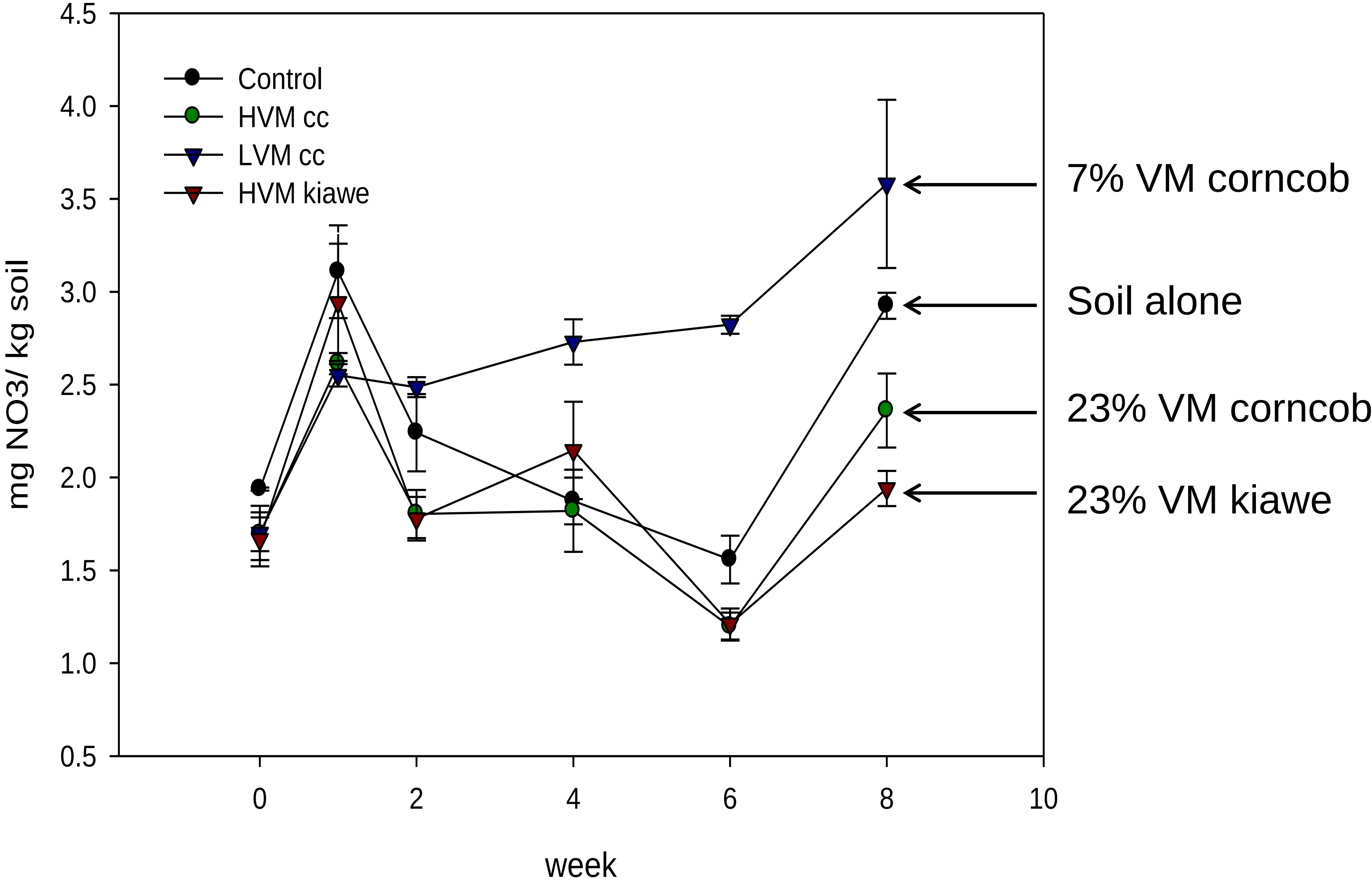
Incubation2: Nitrate nitrogen



Incubation 2: Ammonium Nitrogen



Incubation2: Nitrate nitrogen



2-week incubation—rationale

- 23% corncob charcoals contained extractable compounds, detected by GC-MS
- The removal of this fraction reduced its effect on microbial activity
- Opportunity to determine the bioavailability of the extractable fraction of high VM corncob charcoal
  - Can the extractable carbon compounds support fungal growth and activity?

2-week incubation of inoculated charcoal and charcoal fractions

Inoculum: Charcoal fungus

Fractions of 23% VM corncob char

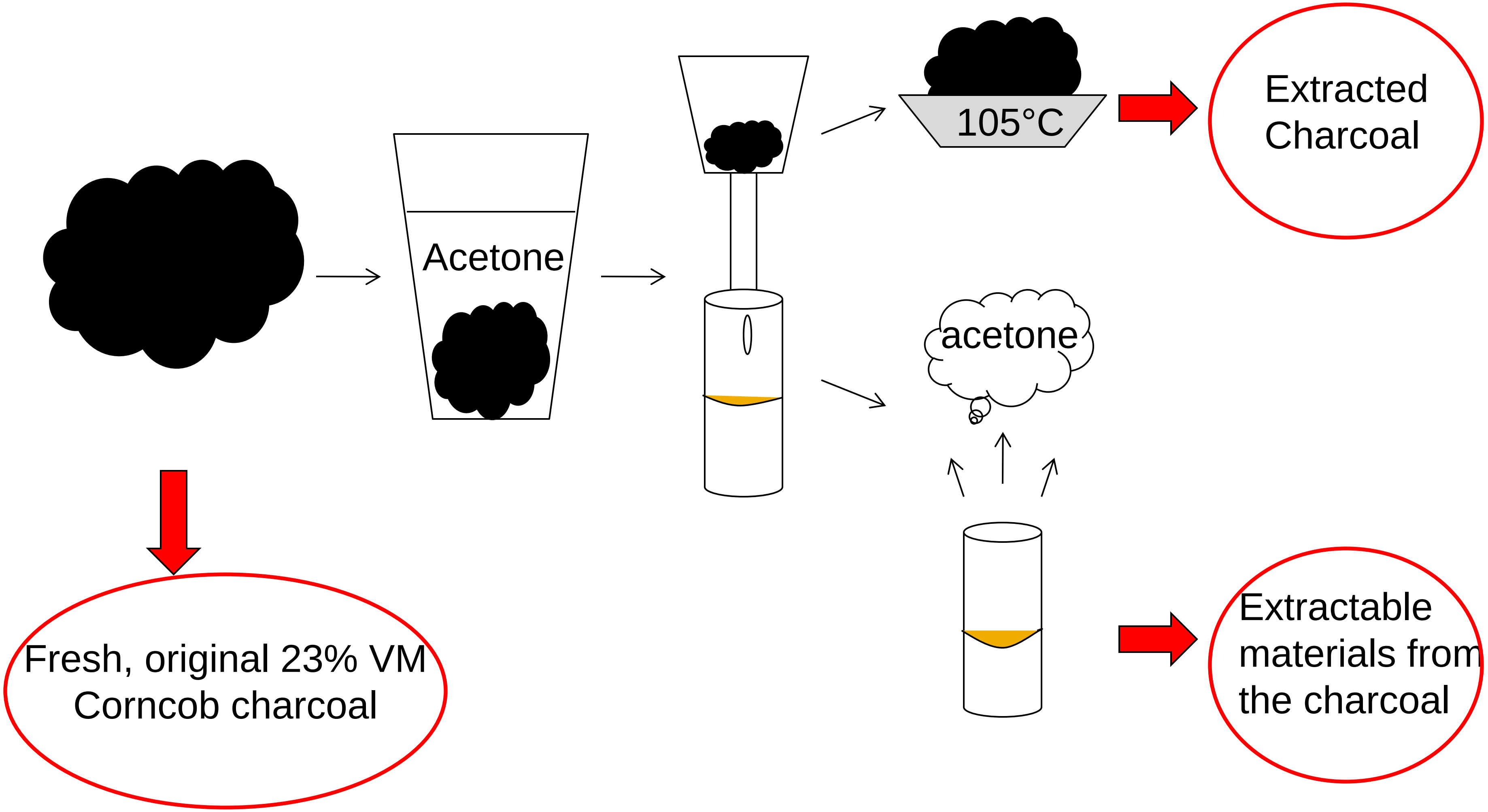
1. Original charcoal
2. Extracted charcoal
3. Extractable components

Nutrition: Fungal nutrient solution

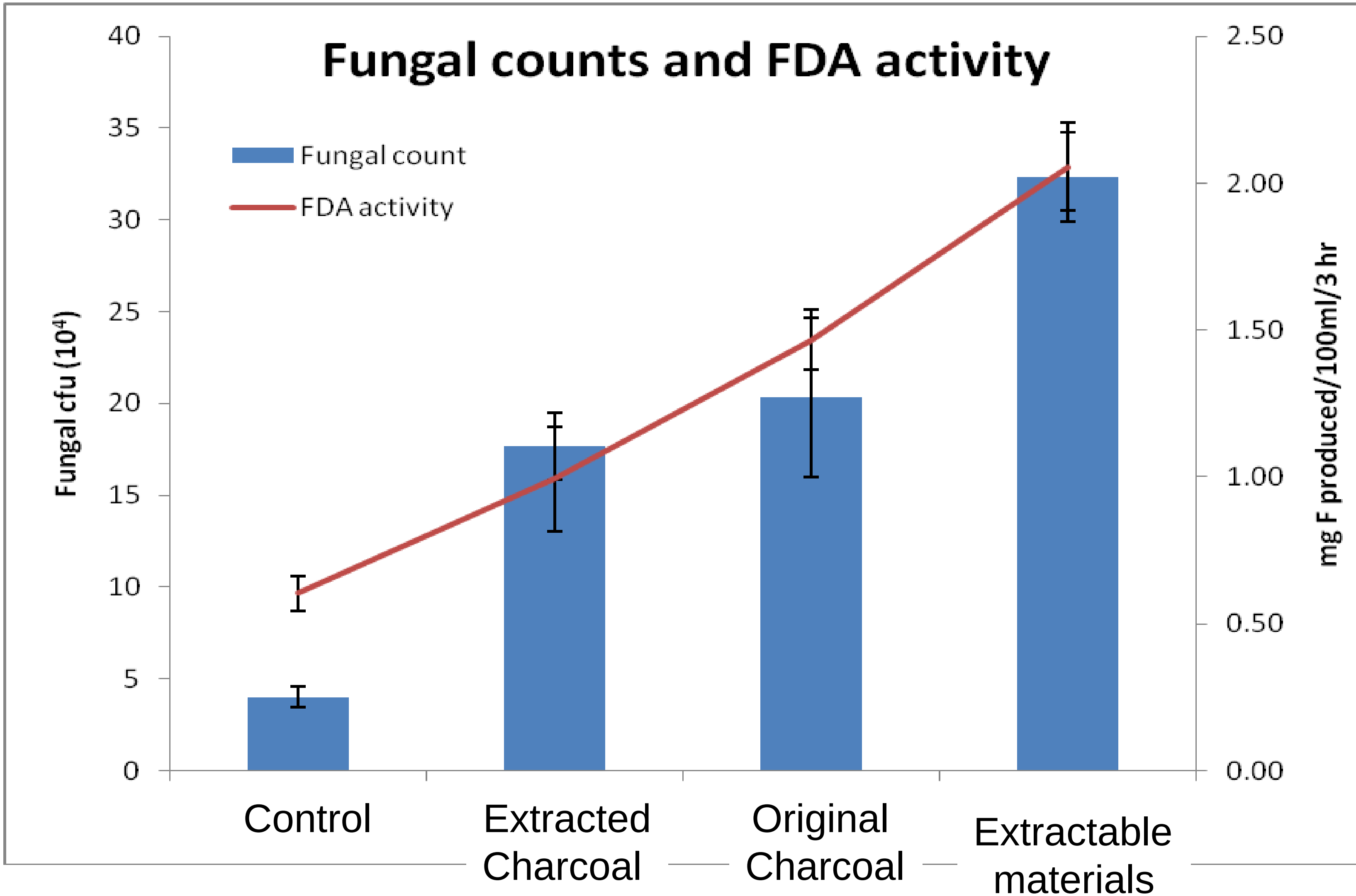
Measurements

1. Fungal colony forming units
2. Hydrolytic enzyme activity

Fractions of 23% VM corncob charcoal



Incubation 3: Fungal Growth and Activity



Interpretation—Study #2

- High VM corncob chars stimulated greater microbial activity than low VM corncob
- High VM kiawe charcoal did **not** enhance microbial activity
- For a single feedstock, VM content provides information regarding its relative bioavailability

  BUT

  Characterization of charcoal's soluble chemical composition with GC-MS was a better predictor of the behavior of charcoal in soil
- Further research using more sensitive techniques for more complete molecular composition of VM content

Study #3: Effect on Soil Charge

Objectives

1. To determine the effect of different charcoals on soil charge in aging experiments.
2. To relate findings to previous studies showing an enhancement of soil CEC.

Hypotheses

- Fresh charcoal exhibits variable charge and will enhance the soil's CEC upon increasing oxidation.
- Charcoals with a higher VM content will undergo oxidation more rapidly.

Procedure for Study #3

Treatments

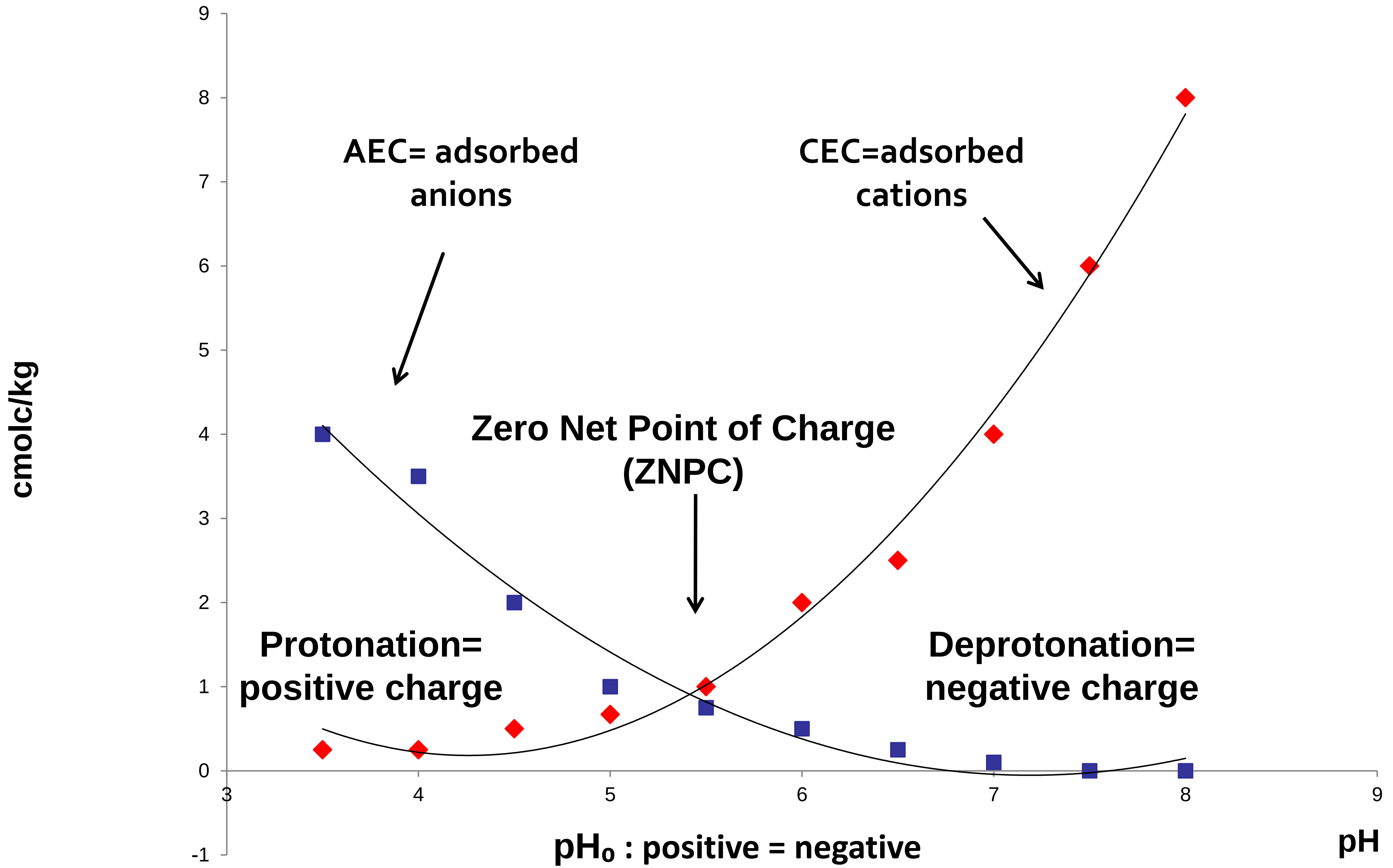
1. 23% VM corncob
   7% VM corncob
   23% VM kiawe
2. Halii Soil and Leilehua Soil
3. Incubated charcoal and charcoal/soil mixtures at 60°C

Charge Fingerprints

1. pH_o
2. Zero net point of charge (ZNPC)
3. Cation exchange capacity (CEC)
4. Anion exchange capacity (AEC)

Incubation with incremental measurements in time

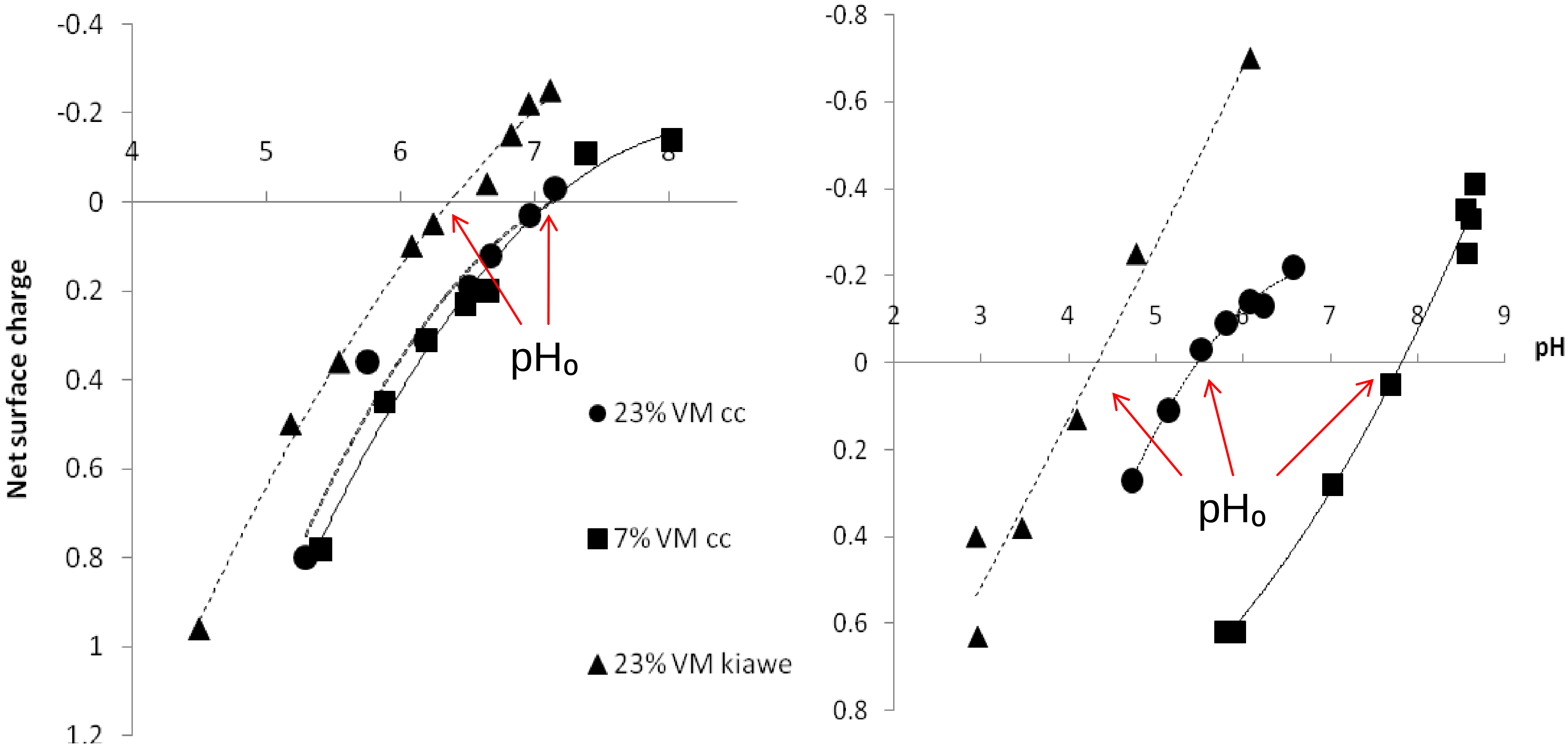
Variable (pH dependent) soil charge



pH_0 : positive = negative

Where there is only variable charge, pH_0= ZNPC

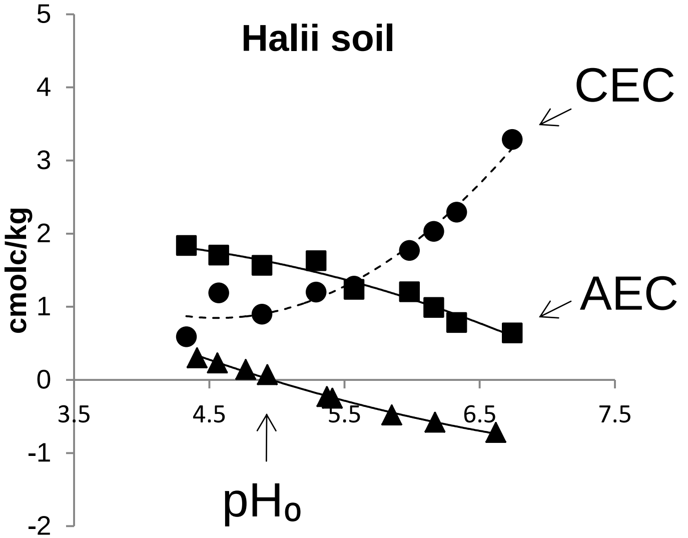
1. Variable Charge of charcoals

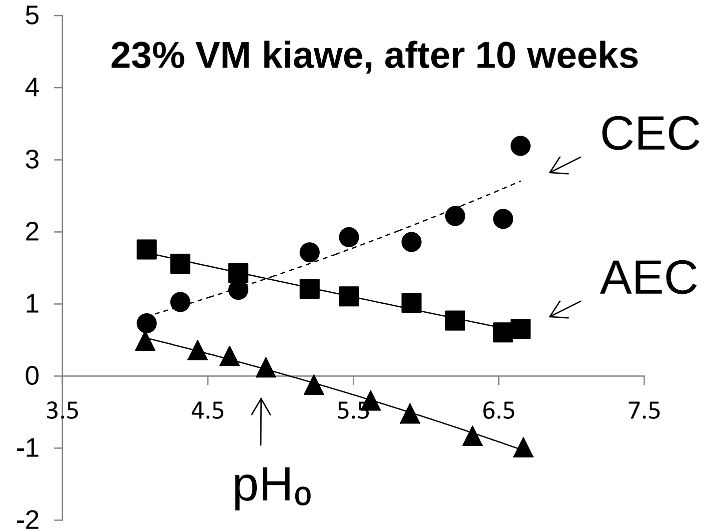


Fresh charcoal

Aged charcoal

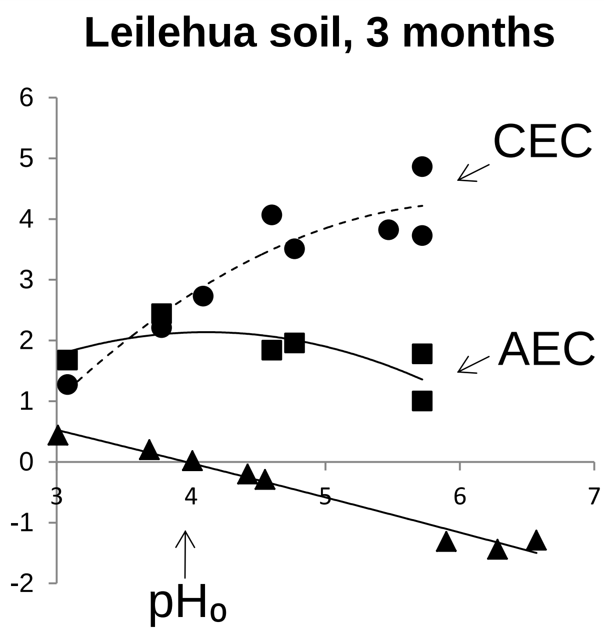
Charcoals + Halii soil

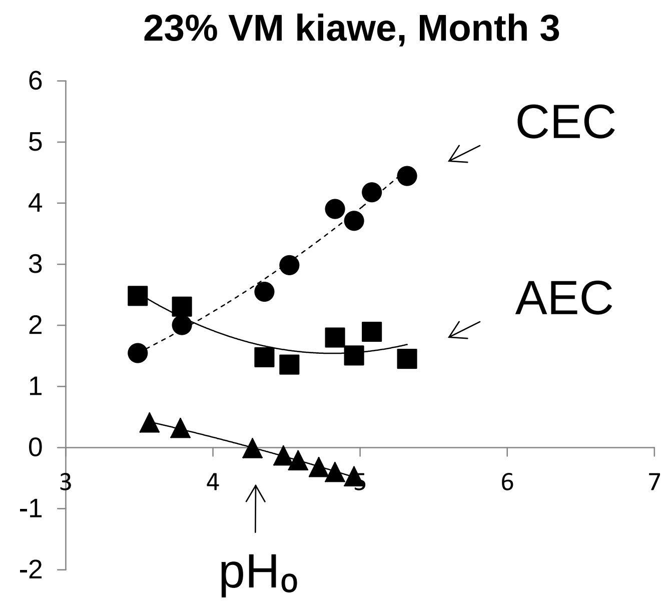




	pH$_0$		ZNPC		CEC pH 7(cmol$_c$ kg^{-1})	
Charcoal	Fresh	Aged-10 wk	Fresh	Aged-10 wk	Fresh	Aged-10wk
7% VM corncob	5.2	5.3	5.53	5.33	3.49	3.71
23% VM corncob	5.09	5.09	5.79	5.34	3.23	4.12
23% VM kiawe	5.07	5.22	5.5	5.15	3.61	3.32
Halii soil	4.96		5.57		3.31	

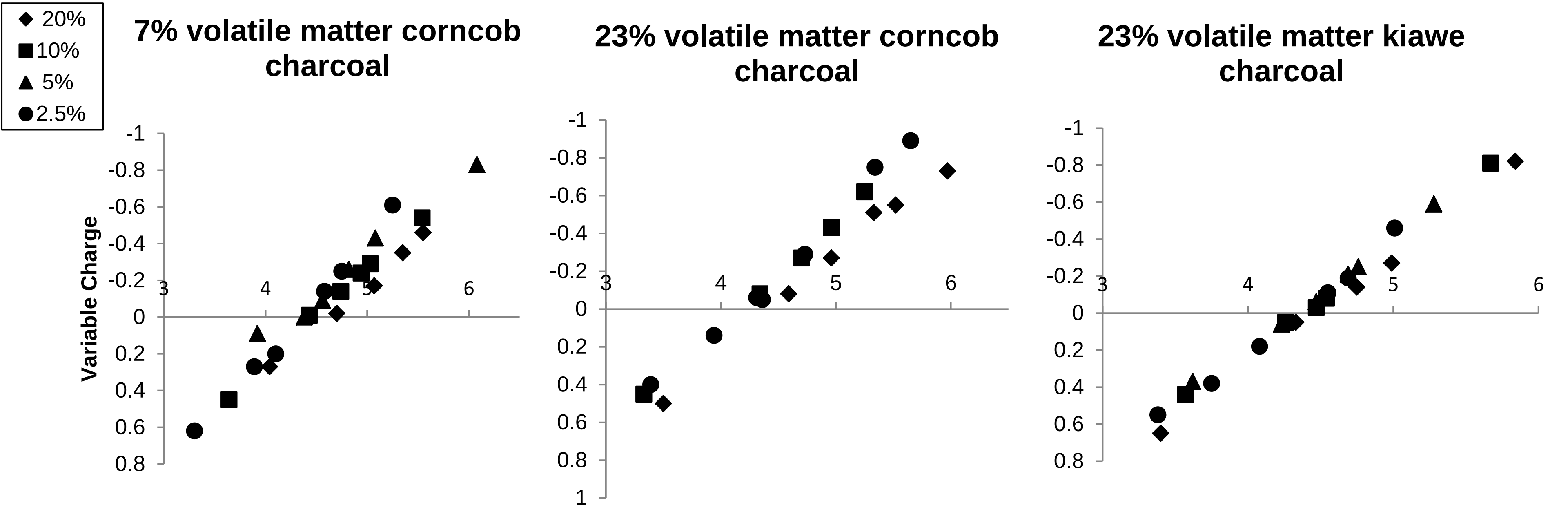
Charcoal/Leilehua Mixtures





	pH_o		ZNPC		CEC pH 7 ($cmol_c\ kg^{-1}$)	
Charcoal	Fresh	Aged-3 mo	Fresh	Aged-3 mo	Fresh	Aged-3 mo
7% VM corncob	4.38	4.23	4.21	3.9	9.83	8.31
23% VM corncob	4.18	4.15	4.04	3.67	7.09	8.54
23% VM kiawe	4.38	4.27	4.18	3.87	13.55	12.65
Soil alone	4.23	3.96	3.26	3.54	11.89	7.84

Rates?


20%
10%
5%
2.5%
7% volatile matter corncob charcoal
Variable Charge
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
3
4
5
6
23% volatile matter corncob charcoal
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
3
4
5
6
23% volatile matter kiawe charcoal
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
3
4
5
6

Interpretation of Study #3

- Aged High VM chars developed greater negative charge than the aged Low VM char
- Shifts in ZNPC and pH_0 due to charcoal additions

- But neither High VM nor Low VM chars dramatically increased soil CEC
  - Due to its variable charge? Masking effect?
  - How long before? At which rate could charcoal have a major increase CEC in soils of Hawaii?

Final Conclusions

- VM content is an informative but coarse measurement.
  - Good indicator of the degree of carbonization for one feedstock
  - But does not tell us a lot about bioavailability

- Extractable (soluble) fraction of charcoal provided important data regarding bioavailable carbon compounds.
  - Still need better technique identify complete molecular composition of VM content

- High VM charcoals developed greater negative charge than the low VM charcoal.
- However, contrary to our hypothesis, no charcoal dramatically enhanced the soil's CEC.

The big picture—soil fertility

- Charcoal Characteristic: Just the beginning
  - Complexity of charcoal chemistry
  - Taken an easily measured property and related its behavior (e.g. bioavailability in soil)—but with limitations
- Soil CEC: Implications for Hawaii soils
  - 70% clay=high specific surface
  - Amending "like with like" (e.g. High specific surface, variable charged Hawaii soil with high specific surface, variable charged charcoal)

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Charge summary

Halii Soil

- Charcoal extracts decreased the ZNPC versus control
- Charcoals had lesser ZNPC than control, except for week 5
- Charcoals had a greater pHo than control for all weeks
- Charcoals had a greater ZNPC than pHo, expect for week 10 of kiawe charcoal
- LVM extracts had greater pHo than LVM char. HVM extracts had same pHo than HVM char. K extracts had slightly greater pHo than char.
- ZNPC for extracts was generally less than or equal to the charcoal ZNPC, except for LVM baseline, K baseline and K 10 week

Leilehua Soil

- HVM ZNPC more negative month 2 and 3. No change in pH_0
- LVM ZNPC more negative month 1, 2, 3. Slight decrease in pH_0
- Kiawe ZNPC more negative month 1, 2, 3. Slight decrease in pH_0
- Soil alone ZNPC slight increase month 1, 2, 3. Decrease in pH_0
- Charcoals always greater pH_0 than control throughout incubation (except HVM baseline)
- Charcoals always greater ZNPC than control, except HVM and LVM month 2
- Charcoals almost 1 pH unit greater ZNPC than control at baseline

Halii Soil

	charcoal									
	HVM corncob			LVM corncob			HVM kiawe			Halii soil
	0	5	10	0	5	10	0	5	10	
pH_0	5.05	5.19	5.06	4.97	5.22	5.15	5.01	5.14	5.1	4.96
ZNPC		5.9	5.42	5.22	6.23	5.33	5.13	5.77	4.9	5.57
ZNPC-pH0		0.71	0.36	0.25	1.01	0.18	0.12	0.63	-0.2	0.61
pH -ctrl		0.23	0.1	0.01	0.26	0.19	0.05	0.18	0.14	0
ZNPC-ctrl		0.33	-0.15	-0.35	0.66	-0.24	-0.44	0.2	-0.67	0
	charcoal/water extract									
	HVM corncob			LVM corncob			HVM kiawe			Halii soil
	0	5	10	0	5	10	0	5	10	
pH_0	5.09	5.17	5.09	5.2	5.37	5.3	5.07	5.18	5.22	4.96
ZNPC	5.79	4.91	5.34	5.54	4.95	5.33	5.5	5.06	5.15	5.57
ZNPC-pH0	0.7	-0.26	0.25	0.34	-0.42	0.03	0.43	-0.12	-0.07	0.61
pH -ctrl	0.13	0.21	0.13	0.24	0.41	0.34	0.11	0.22	0.26	0
ZNPC-ctrl	0.22	-0.66	-0.23	-0.03	-0.62	-0.24	-0.07	-0.51	-0.42	0
char-extract pHo	-0.04	0.02	-0.03	-0.23	-0.15	-0.15	-0.06	-0.04	-0.12	
char-extract ZNPC		0.99	0.08	-0.32	1.28	0	-0.37	0.71	-0.25	0

Leilehua Soil

	HVM corncob				LVM corncob				HVM kiawe				Soil alone			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
pH_0	4.18	4.15	4.18	4.15	4.38	4.27	4.29	4.23	4.38	4.25	4.26	4.27	4.23	4.04	4.05	3.96
ZNPC	4.04	4.07	3.47	3.67	4.21	3.51	3.42	3.9	4.18	3.57	3.69	3.87	3.26	3.33	3.46	3.54

Difference	-0.14	-0.08	-0.71	-0.48	-0.17	-0.76	-0.87	-0.33	-0.2	-0.68	-0.57	-0.4	-0.97	-0.71	-0.59	-0.42
pH-ctrl	-0.05	0.11	0.13	0.19	0.15	0.23	0.24	0.27	0.15	0.21	0.21	0.31				
ZNPC-ctrl	0.78	0.74	0.01	0.13	0.95	0.18	-0.04	0.36	0.92	0.24	0.23	0.33				

Characterization

- 130-190°C: cellulose and lignin begin to decompose
- >200C carbonization begins, nitrogen volatilizes, 760 C to vaporize K and P
- **Cellulose** and pectin: up to 200 C, complex glucans form
  - >270 C, new aliphatic structures detected by NMR: aliphatic C, phenol and/or furan C, aromatic C, and carbonyl C
  - From carbohydrate char at 250 C to being dominated by phenols, furans, aromatic C at 350 C
  - >300, furan like compound products. Condensation sites: nonvolatiel anhydroglucose cores in side chains…glue for nonvolatile residues … more heating=furan polymer to aromatic polymer
  - PAHs detected when heated 300-600C Diels Alder cycloaddition
  - **Pectin** oxidized at 550C totally
  - Change in volatile compositionL furans, pyronones, anhydrousugars and furfural at low T to phenol, catechol, and subst-phenols at high T
  - At 200 C: NMR detected aliphatic, O-aryl, aryl, carbonyl, further heting =depolymerization and greater aromatic C with lesser amounts of ketones and aliphatic C at 550C
- Lignin
  - Common monomeric units of polymer=phenylpropanol linked by ether bond or C-C
  - Heating lignon yields volatiles, mostly methoxyphenols=highest yield between 500 and 600 C
  - 40-60% volatilized at 450C and 740C
  - Heating from 250 to 400C diminised phenolic C and alcoholic C yields and removed aromatic substitutions
  - Fused ring formation at 400-500C
  - Dehydration reactions predominate at lower T and decarboxylation at highT

Proteins: decompose systematic and random depolymerization rxns

Tar = defined as the VOLATILES?

Overall reactions: Lower T dehydation, dehydrogenation and degradation of O-alkyl C

350C, more stable alkyl C and carboxyl C is removed and only part of recalcitrant, cyclic, or branched paraffinic structures remain in a condensed matrix, and increase in aryl C. Aryl:alkyl ratio as index of charring?

Paraffinics and sterols are more stable than fatty acids (GCMS)

Synthesis of new aromatic CL demethoxylation as decresing methoxyphenol:phenol ratio

350C: atomic C:O ratio=3 (Baldock and Smernik, 2002), shows that furans, anhydrosugars and structures from cellulose are important part of char

Atomic C to H=almost every second aromatic C is connected to H

GCMS

- Raw kiawe=contained molecules typical of plant material
  - Straight chain aromatic= octacosane. Nonacosane=fuel; sterols-plant cholesterols
- Much less in corncob feedstock but typical plant materials
- 23% VM corncob
  - Myristic aldehyde: fatty acid derivative? Benzene ring, ether bonds, C=O
  - Dimethylethyl phenol: phencol with C-C, and OH
  - Dodecyl acrylate: alkyl chain with oxygen substition (ester bond C=C=O and C-O-C
  - Oleamide: amide of fatty acid oleic acid
  - Propanoic acid:HO-C=O and C-C-C
- 63% VM corncob
  - Ethyl phenol: phenol with ethyl group
  - Ethyl guaiacol: phenol with ethyl group and ether group
  - Vinyl guaiacol: pyrolysis product of lignin: phenol with ether group and C=C
  - Syringol: phenol with two ether groups C-O-C
  - Vanillin: aldehyde ether phenol, O=C-H
  - Tridecanol: CH chain with OH at end
  - Dodecyl acrylate: CH chain and C=C-C=O and C-OC (ester)
  - Methyloxyphenol: phenol with ether
  - Palmitic acid: fatty acid CH chain with carboxylic groups at end
  - FluoratheneL: PAH naphthalene or benzene stuck together with 5 member ring
  - Pyrene: 2 benzene and 2 two 6 member ring each with a double bond

Significant for incubations-1 mo

FDA	P-value
Treatment	<0.0001
Time	<0.0001
Nitrogen	0.2461
Treatment*time	<0.0001
Nitrogen*time	<0.0001
Ammonium	
Treatment	<0.0001
Time	<0.0001
Nitrogen	<0.0001
Treatment*time	<0.0001
Nitrogen*time	<0.0001
Nitrate	
Treatment	<0.0001
Time	<0.0001
Nitrogen	<0.0001
Treatment*time	<0.0001
Nitrogen*time	<0.0001

FDA	P-value
HVM > LVM and Control	<0.0001
LVM > Control	0.7457
HVM+N > LVM+N and Control+N	<0.0001
LVM+N > Control+N	0.9277
HVM+N > HVM	<0.0001
LVM+N > LVM	0.6012
Control+N > Control	0.7712
Ammonium	
HVM < LVM and Control	<0.0001
LVM < Control	0.1426
HVM+N < LVM+N and Control+N	<0.0001
LVM+N < Control+N	0.0001
HVM+N > HVM	<0.0001
LVM+N > LVM	<0.0001
Control+N > Control	<0.0001
Nitrate	
HVM > LVM and Control	0.0268
LVM > Control	0.2803
HVM+N < LVM+N and Control+N	<0.0001
LVM+N < Control+N	<0.0001
HVM+N > HVM	<0.0001
LVM+N > LVM	<0.0001
Control+N > Control	<0.0001

FDA	P-value
HVM > LVM and Control	Significant at Day 3 and persisted for remainder of experiment
LVM > Control	Not significant for entire experiment
HVM+N > LVM+N and Control+N	Significant at Day 3 and persisted for remainder of experiment
LVM+N > Control+N	Not significant for entire experiment
HVM+N > HVM	Significant at Day 3 and persisted for remainder of experiment
LVM+N > LVM	Not significant for entire experiment
Control+N > Control	Not significant for entire experiment
Ammonium	
HVM < LVM and Control	Significant at Day 7 and for remainder of experiment, except for Day 14
LVM < Control	Not significant for entire experiment
HVM+N < LVM+N and Control+N	Significant at Day 7 and persisted for remainder of experiment
LVM+N < Control+N	Significant for entire experiment with exceptions of Days 3 and 10
HVM+N > HVM	Significant for entire experiment
LVM+N > LVM	Significant for the entire experiment
Control+N > Control	Significant for entire experiment
Nitrate	
HVM < LVM and Control	Significant at Days 7, 21, 28
LVM < Control	Not significant for entire experiment
HVM+N < LVM+N and Control+N	Significant at Day 7 and for remainder of experiment
LVM+N < Control+N	Significant at Day 7 and for remainder of experiment
HVM+N > HVM	Significant for the entire experiment
LVM+N > LVM	Significant for the entire experiment
Control+N > Control	Significant for the entire experiment

WEC:
Time 0 HVM and others <.0001; Lvm and ctrl <.0001; HVM and HVMN 0.7917
Time 1: HVM and others <.0001; Lvm and ctrl 0.0004; HVM and HVMN 0.2896
Time 3: HVM and others 0.0826; Lvm and ctrl 0.0006; HVM and HVMN 0.4520
Time 7: HVM and others 0.7126; Lvm and ctrl 0.0002; HVM and HVMN 0.1090
Time 10:HVM and others 0.4403; Lvm and ctrl <.0001; HVM and HVMN 0.2838
Time 14: HVM and others 0.0497; Lvm and ctrl 0.0004;HVM and HVMN 0.5453
Time 21: HVM and others 0.9057; Lvm and ctrl 0.0065 HVM and HVMN 0.8519
Time 28: HVM and others 0.5970; Lvm and ctrl <.0001 HVM and HVMN 0.2322

Significant for incubations-2 mo

- FDA (autoregressive heterogeneous Proc Mixed)
- Overall: Time; Txt; Time*Txt = p<0.001
- Time 0 txt:p<0.001
  - ctrl v H-Ch 0.9529
  - H-Ch v L-Ch 0.5368
  - H-Ch v K-Ch 0.7307
  - H-Ch v H-ext 0.4690
- Time 1 wk: p<0.001
  - ctrl v H-Ch : 0.0086
  - H-Ch v L-Ch : 0.0020
  - H-Ch v K-Ch :0.8567
  - H-Ch v H-ext: 0.0059
- Time 2 wk: p<0.001
  - ctrl v H-Ch :0.0006
  - H-Ch v L-Ch: 0.0037
  - H-Ch v K-Ch :0.5819
  - H-Ch v H-ext : 0.0382
- Time 4 wk:
  - ctrl v H-Ch: <.0001
  - H-Ch v L-Ch: <.0001
  - H-Ch v K-Ch : 0.3359
  - H-Ch v H-ext : 0.0009
- Time 6 wk
  - ctrl v H-Ch : 0.0138
  - H-Ch v L-Ch : 0.0545
  - H-Ch v K-Ch : 0.1279
  - H-Ch v H-ext : 0.9378
- Time 8 wk
  - ctrl v H-Ch : 0.0099
  - H-Ch v L-Ch : 0.0010
  - H-Ch v K-Ch : 0.2072
  - H-Ch v H-ext : 0.7869

- NH4 (autoregressive heterogeneous Proc Mixed)
- Overall: Time; Txt; Time*Txt = p<0.001
- Time 0 txt:p<0.001
  - ctrl v H-Ch 0.5913
  - H-Ch v L-Ch 0.7598
    H-Ch v K-Ch 0.4603
    H-Ch v H-ext 0.4390
- Time 1 wk: p 0.0044
  - ctrl v H-Ch 0.0245
    H-Ch v L-Ch 0.3462
    H-Ch v K-Ch 0.1195
    H-Ch v H-ext 0.5963
- Time 2 wk: p<0.001
  - ctrl v H-Ch 0.0091
    H-Ch v L-Ch 0.2547
    H-Ch v K-Ch 0.1064
    H-Ch v H-ext 0.6955
- Time 4 wk:
  - ctrl v H-Ch 0.0003
    H-Ch v L-Ch 0.4346
    H-Ch v K-Ch 0.0604
    H-Ch v H-ext 0.1297
- Time 6 wk
  - ctrl v H-Ch <.0001
    H-Ch v L-Ch 0.1199
    H-Ch v K-Ch 0.0130
    H-Ch v H-ext 0.1018
- Time 8 wk
  - ctrl v H-Ch <.0001
    H-Ch v L-Ch <.0001
    H-Ch v K-Ch <.0001
    H-Ch v H-ext 0.0601

- NO3(autoregressive heterogeneous Proc Mixed)
- Overall: Time; Txt; Time*Txt = p<0.001
- Time 0 txt:p<0.001
  - ctrl v H-Ch 0.0444
  - H-Ch v L-Ch 0.9516
    H-Ch v K-Ch 0.7650
    H-Ch v H-ext 0.0546
- Time 1 wk: p0.13
  - ctrl v H-Ch 0.0535
    H-Ch v L-Ch 0.7974
    H-Ch v K-Ch 0.1185
    H-Ch v H-ext 0.7178
- Time 2 wk: p<0.001
  - ctrl v H-Ch 0.0328
    H-Ch v L-Ch 0.0020
    H-Ch v K-Ch 0.0015
    H-Ch v H-ext 0.9921
- Time 4 wk:
  - ctrl v H-Ch 0.7976
    H-Ch v L-Ch 0.0003
    H-Ch v K-Ch 0.0096
    H-Ch v H-ext 0.2979
- Time 6 wk
  - ctrl v H-Ch 0.0017
    H-Ch v L-Ch <.0001
    H-Ch v K-Ch <.0001
    H-Ch v H-ext 0.5777
- Time 8 wk
  - ctrl v H-Ch 0.1143
    H-Ch v L-Ch 0.0021
    H-Ch v K-Ch 0.0001
    H-Ch v H-ext 0.7300

Significance for 2-wk and Prussian Blue

- FCU & FDA
  - Sqrt transformed
  - Acetone extractable=A
  - 23% VM char=B
  - Extracted char=C (FDA) and B (counts)
  - Control= D (FDA) and C(counts)
- Prussian blue
  - Log transformed for to pass equal variance
  - HVM chars=A
  - Extracted=B
  - 7%VM cc =C

Mineralization slope

- Soil alone=A
- All charcoals =B

Discussion of techniques

- GCMS: control set chamber to 250C. GC separates molecules by their partitioning properties in the columns. MS then charges ions. It identifies then by their mass to charge ratio. The time it takes to travel through the system is a function of its molecular weight
- FTIR: collects infrared spectra. Shoots out infrared energy which certain frequencies are absorbed by molecules, which is characteristic of their structure. So, absorption=vibrational frequency. Different types of vibrations, known as vibration modes. Absorption bands: along wavenumbers—triple bonds, 2300 -2100; double, 1800-1500; and single (fingerprint) 1500 -1000.
- NMR: emit a magnetic pulse, nuclei absorb energy and radiates energy back at a specific resonance frequency. Two steps: (1) apply constant magnetic field and align or polarize magnetic nuclear spins (2) perturbation with pulse to observe nuclei. You can gain structural information about molecular groups by observing their shifts on the resonant frequency of the nuclei present

- **2.2.2 FTIR**
- An FTIR-attenuated total reflectance (ATR) technique was employed to qualitatively analyze the functional groups in the 23% and 7% volatile matter corncob charcoals, 23% volatile matter kiawe charcoal, and the activated charcoal samples. Spectra were recorded within the 4,000 cm^{-1} to 400 cm^{-1} range with a resolution of 16 cm^{-1} by obtaining 256 scans on a Thermo Nicolet 380 spectrometer with the Smart Performer accessory. The interpretation of the FTIR spectra were intended for qualitative analysis only.
- **2.2.3 NMR**
- We used a 200 MHz Bruker DSX spectrometer (^{13}C frequency 50 MHz) equipped with a 4mm magic angle spinning (MAS) probe to obtain ^{13}C NMR spectra of the 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoals. All analyses were conducted at a rotor spinning rate of 7 kHz. We improved quantitation with a relatively high spinning rate, which moves the spinning sideband signals outside of the ^{13}C chemical shift range (i.e. 0 – 220 ppm) and prevents overlap with other signals. Cross polarization (CPMAS) were acquired by applying a 90 degree ^{1}H excitation pulse, 1ms ^{13}C contact pulse, two-pulse phase-modulated (TPPM) ^{1}H decoupling, and a 3 s recycle delay between scans.
- The highly aromatic structure of charcoal makes quantitative characterization of charcoals by CPMAS NMR difficult since it inhibits efficient ^{1}H-^{13}C polarization transfer. Thus, CPMAS NMR spectra are best regarded as semi-quantitative. We overcame this limitation by using a direct polarization pulse sequence (DPMAS). We utilized a 20 degree excitation pulse, which reduced the recycle delay from 100 s to 5 s and increased the signal-to-noise per unit time by a factor of 16 relative to spectra acquired with a 90 degree excitation pulse. We acquired DPMAS spectra with ^{1}H-^{13}C dipolar-dephasing by inserting a 50 µs dephasing delay prior to the TPPM decoupling, which are devoid of signals from carbon atoms with a directly bonded hydrogen atom (C-H). Due to the behavior of aromatic model compounds, we applied a 10% intensity correction to signals in the dipolar-dephasing NMR spectra to compensate for relaxation during the 50 µs delay.
- We subtracted background signals arising from carbon-containing probe and rotor components from each of the charcoal spectra so that we could quantitatively interpret the peak areas obtained by DPMAS as measurements for carbon functional groups. The quantitative reliability of all NMR experiments has been assessed by calculating (see Equation 1) the percentage of sample carbon observed in the spectrum (C_{obs}), using a procedure known as spin counting (Smernik & Oades; 2000). (1

Charcoal Structure Elucidation

- We used a novel ^{13}C-^{1}H dipolar dephasing technique based upon the DPMAS sequence (described above) for a quantitative measure of the protonated versus the bridgehead aromatic carbons within the charcoal backbone structure. We estimated the average the number of aromatic carbon atoms fused together in a cluster, average number of oxygen atoms per cluster, and the average number and length of the alkyl side chains attached to each cluster of aromatic carbons, using the algorithms derived by Solum et al., (1989).
- **2.2.4 GC-MS**
- The chemical analysis was performed by GC-MS for the 63%, 23%, 7% VM and acetone-extracted corncob charcoal; 23% VM kiawe charcoal; activated charcoal; and raw corncob husk and kiawe wood. We extracted 1-g of each sample by sonication with acetone for 30 min. The extracts were filtered then analyzed with a Varian CP-3800 gas chromatograph interfaced with a Varian 1200 mass spectrometer. A Factor Four VF5-MS (Varian) capillary column was used. The GC-MS ion source and transfer lines were kept at 200 and 250°C respectively, and the analysis was conducted in electron impact at 70 eV, full scan mode (50-550 u range). The NIST 2002 mass spectral library was used for compounds mass spectral identification.
- **2.2.5 Prussian Blue for Phenols**
- Prussian blue analysis to measure total phenol content followed the protocol outlined by Stern et al. (1996). Samples of 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoals were extracted with 90% acetone, in triplicate. One hundred microliters of the extract from each sample was transferred into 30-ml test tubes. Three ml of ferric ammonium sulfate (0.1 *M* $FeNH_4(SO_4)_2$ in 0.1 *M* HCl) was then added to successive samples at 1.0 minute intervals. Exactly 20 minutes after the ferric ammonium sulfate additions, 3.0 ml of potassium ferricyanide (0.008 *M* $K_3Fe(CN)_6$) was added to each sample, successively. Exactly 20 minutes later, the absorbance was read at 720 nm.