

# Supplementary Information 2: Lipid residues in pottery from the Indus Civilisation in northwest India

Suryanarayan et al. 2020

## R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see <http://rmarkdown.rstudio.com>.

```
#Plots for Suryanarayan et al. 2020
```

## Load packages

```
library(readr)
library(ade4)
library(ggplot2)
library(knitr)
library(tidyr)
library(reshape2)
```

```
##
## Attaching package: 'reshape2'

## The following object is masked from 'package:tidyr':
##
## smiths
```

```
library(RColorBrewer)
```

```
#Load data
```

```
data <- read_csv("PhD/Data/data.csv")
```

```
##
## -- Column specification -----
## cols(
##   .default = col_character(),
##   Rim_diam_cm = col_double(),
##   Sample_mass_mg = col_double(),
##   Area_GC_total = col_double(),
##   AreaC12 = col_double(),
##   AreaC14 = col_double(),
```

```

## AreaC15 = col_double(),
## AreaC161 = col_double(),
## AreaC16 = col_double(),
## AreaC17 = col_double(),
## AreaC181 = col_double(),
## AreaC18 = col_double(),
## AreaC19 = col_double(),
## AreaC201 = col_double(),
## AreaC20 = col_double(),
## AreaC21 = col_double(),
## AreaC221 = col_double(),
## AreaC22 = col_double(),
## AreaC23 = col_double(),
## AreaC24 = col_double(),
## AreaC25 = col_double()
## # ... with 24 more columns
## )
## i Use 'spec()' for the full column specifications.

```

```
save(data, file = "data.RData")
```

#Prepare data ## subset data and set order of sites

```

data_refs <- droplevels(subset (data, (Site_name == "MSDI") | (Site_name == "LHRI") |
  (Site_name == "FRN") | (Site_name == "MSDVII") |
  (Site_name == "RGR") | (Site_name == "KNK") |
  (Site_name == "ALM" |
    (Site_name == "Tamil Nadu cow milk") |
    (Site_name == "Gujarat milk pot"))))

data_refs$Site_name <- factor(data_refs$Site_name, levels = c("ALM", "MSDVII",
  "MSDI", "LHRI", "KNK", "FRN",
  "RGR",
  "Tamil Nadu cow milk",
  "Gujarat milk pot"))

```

#convert variables to factors

```

data$Site_name <- factor(data$Site_name)
data$Vessel_type <- factor(data$Vessel_type)
data$Rural_urban <- factor(data$Rural_urban)

```

subset rural and urban sites & set order of sites

```

data_rural <- droplevels(subset (data, (Rural_urban == "Rural")))
data_rural$Site_name <- factor(data_rural$Site_name, levels = c("ALM", "MSDVII",
  "MSDI", "LHRI", "KNK"))

data_urban <- droplevels(subset (data, (Rural_urban == "Urban")))
data_urban$Site_name <- factor(data_urban$Site_name, levels = c("FRN", "RGR"))

```

```
#subset to NW India settlements
```

```
indus <- droplevels(subset(data, (Site_name=="RGR") | (Site_name=="MSDI") |  
                             (Site_name=="LHRI") | (Site_name=="MSDVII") | (Site_name=="ALM")  
                             | (Site_name=="FRN") | (Site_name=="KNK"))) )  
isotope_indus <- droplevels(subset(indus, !is.na(indus$delta13C_C16)))
```

```
#convert variables to factors
```

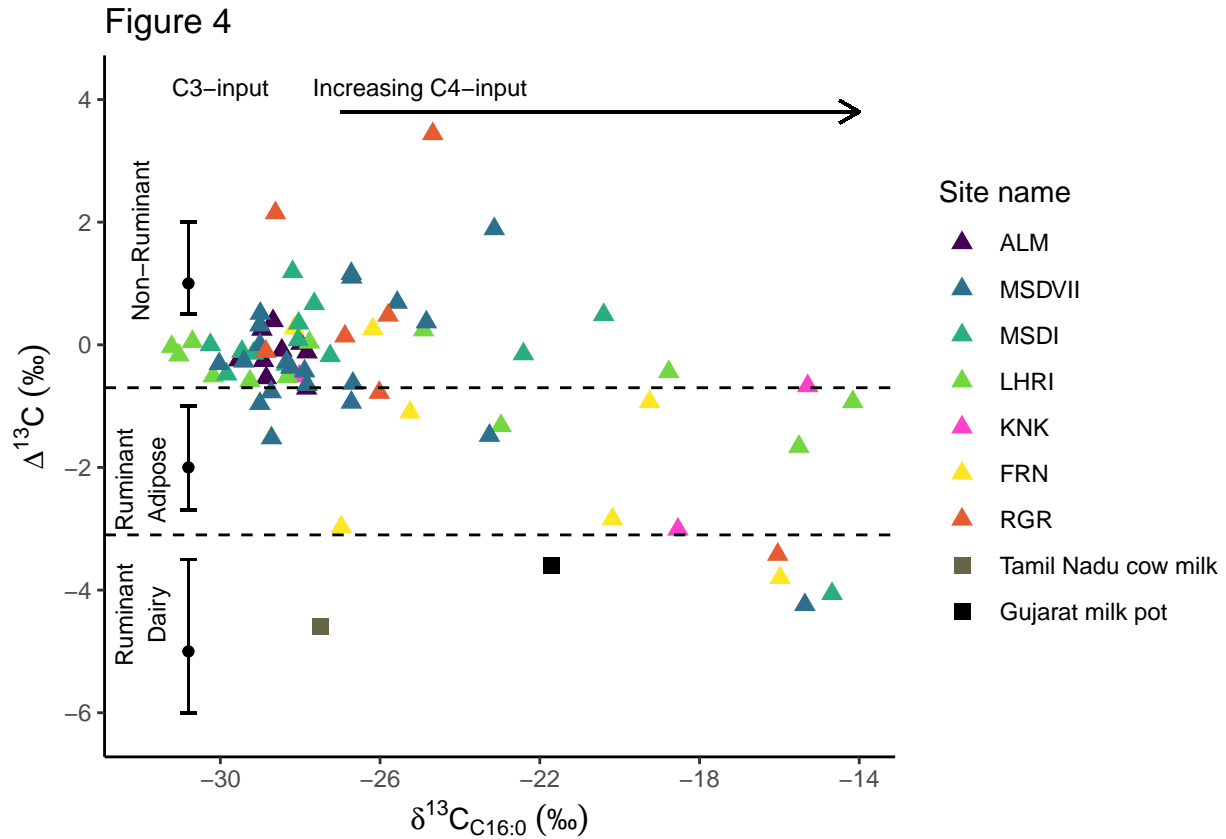
```
isotope_indus$Vessel_type <- factor(isotope_indus$Vessel_type)  
isotope_indus$Site_name <- factor(isotope_indus$Site_name)
```

```
#Figure 4
```

```
fig4 <- ggplot(data_refs, aes(x = delta13C_C16, y = bigdelta,  
                             colour = Site_name, shape = Site_name)) +  
  geom_point(size = 2.5) + geom_hline(yintercept = c(-0.7, - 3.1),  
                                     linetype="dashed") +  
  annotate("text", x=-32, y=-4.4, label= "Ruminant\nDairy", size=3, angle=90) +  
  annotate("text", x=-32, y=-2.2, label= "Ruminant\nAdipose", size=3, angle=90) +  
  annotate("text", x=-32, y=1.7, label= "Non-Ruminant", size=3, angle=90) +  
  annotate("text", x=-30, y=4.2, label= "C3-input", size=3, angle=0) +  
  annotate("text", x=-25, y=4.2, label= "Increasing C4-input", size=3, angle=0) +  
  geom_segment(x = -27, y = 3.8, xend = -14, yend = 3.8, size = 0.5,  
              arrow = arrow(length = unit(0.3, "cm")), colour = "black") +  
  expand_limits(x = c(-14, -32), y = c(-6.2, 4)) +  
  scale_y_continuous(breaks=c(-6, -4, -2, 0, 2, 4)) +  
  scale_x_continuous(breaks=c(-34, -30, -26, -22, -18, -14)) +  
  theme(plot.title = element_text(hjust=0, size=16)) +  
  labs(x = expression(delta13* C [C16:0]* " "("\u2030")),  
       y = expression(Delta13* C* " "("\u2030")),  
       colour="Site name", shape = "Site name") + ggtitle("Figure 4") +  
  theme_classic() +  
  scale_colour_manual(name = "Site name",  
                     values = c("#440154FF", "#2D708EFF",  
                                "#29AF7FFF", "#73D840FF", "#ff41ca",  
                                "#FDE725FF", "#e55c30", "#666547",  
                                "black")) +  
  scale_shape_manual(name = "Site name",  
                    values = c(17, 17, 17, 17, 17, 17, 17, 15, 15)) +  
  geom_segment(x = -30.8, y = 2, xend = -30.8, yend = 0.5, colour = "black") +  
  geom_segment(x = -30.8, y = -1, xend = -30.8, yend = -2.7, colour = "black") +  
  geom_segment(x = -30.8, y = -3.5, xend = -30.8, yend = -6, colour = "black") +  
  geom_segment(x = -30.6, y = 2, xend = -31, yend = 2, colour = "black") +  
  geom_segment(x = -30.6, y = 0.5, xend = -31, yend = 0.5, colour = "black") +  
  geom_segment(x = -30.6, y = -1, xend = -31, yend = -1, colour = "black") +  
  geom_segment(x = -30.6, y = -2.7, xend = -31, yend = -2.7, colour = "black") +  
  geom_segment(x = -30.6, y = -3.5, xend = -31, yend = -3.5, colour = "black") +  
  geom_segment(x = -30.6, y = -6, xend = -31, yend = -6, colour = "black") +  
  annotate ("point", x = -30.8, y = 1, colour = "black") +  
  annotate ("point", x = -30.8, y = -2, colour = "black") +  
  annotate ("point", x = -30.8, y = -5, colour = "black")
```

```
fig4
```

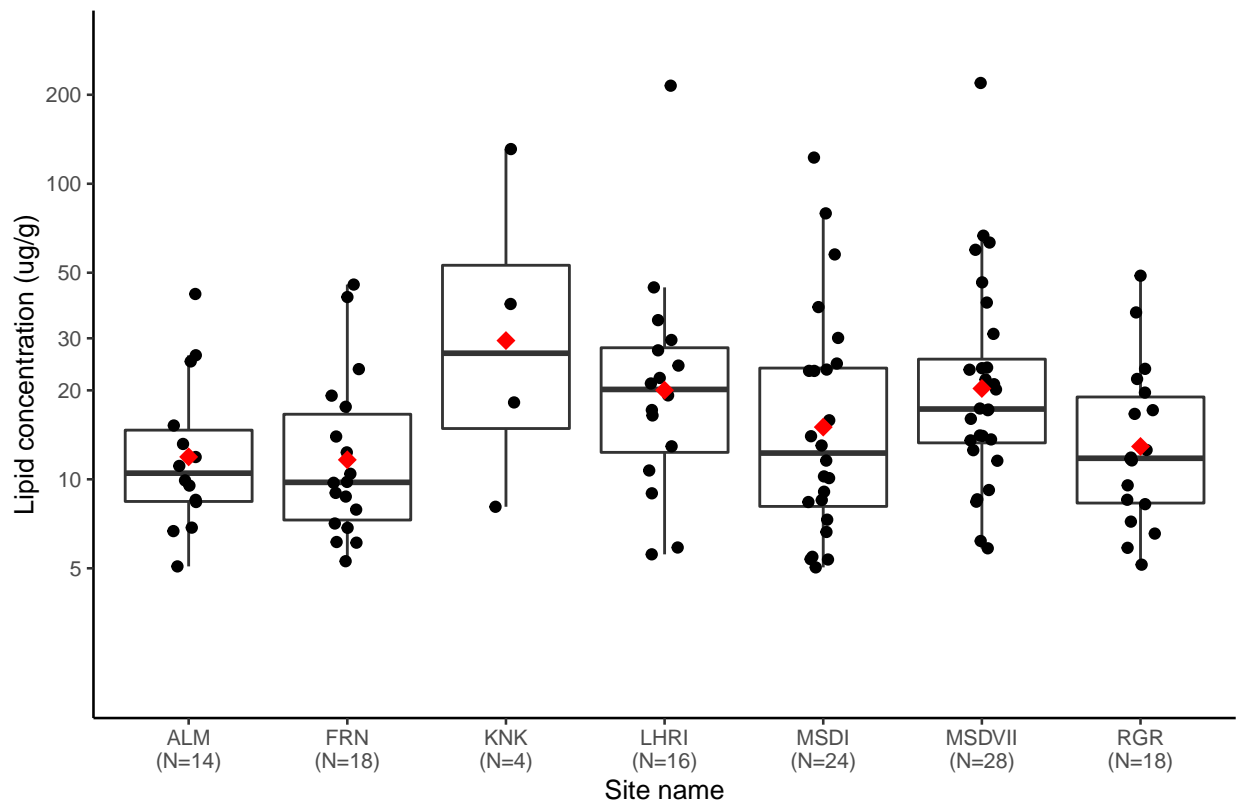
## Warning: Removed 49 rows containing missing values (geom\_point).



#Figure 5

```
fig5 <- ggplot(data = indus, aes(x = Site_name, y = Lipidconc_ug_g)) +
  geom_boxplot(aes(x=Site_name, y=Lipidconc_ug_g),
    position = position_dodge(0.5), width = 0.8, outlier.shape = NA) +
  scale_y_log10(breaks = c(5, 10, 20, 30, 50, 100, 200),
    limits = c(2, 300)) +
  xlab("Site name") + ylab("Lipid concentration (ug/g)") + ggtitle("Figure 5") +
  geom_jitter(aes(y = Lipidconc_ug_g), position = position_jitter(0.1)) +
  stat_summary(fun = mean, geom = "point", shape = 18, size = 3, color = "red") +
  scale_x_discrete(labels = paste(levels(indus$Site_name),
    "\n(N=", table(indus$Site_name), ")", sep = "")) +
  theme_classic(base_size = 10)
fig5
```

Figure 5



#Figure 6a

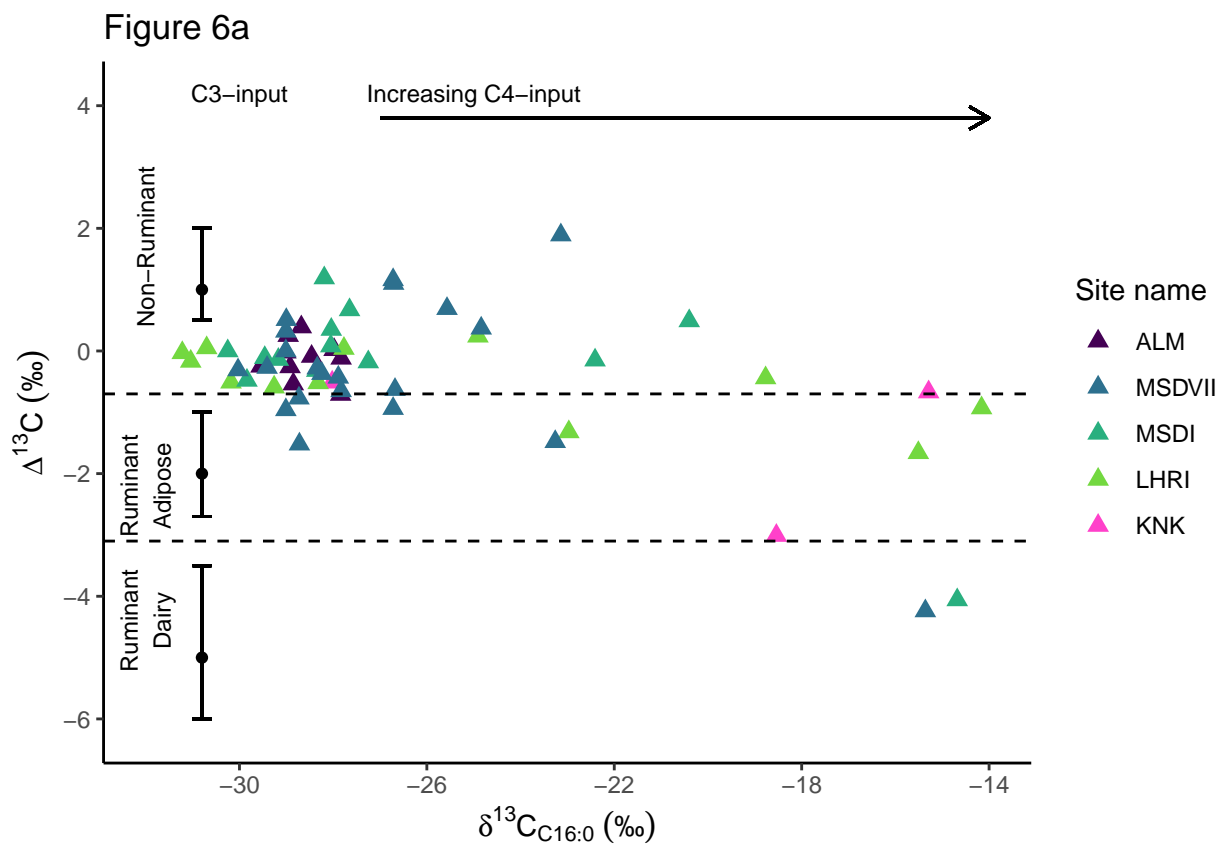
```
fig6a <- ggplot(data_rural, aes(x = delta13C_C16, y = bigdelta,
                                colour = Site_name, shape = Site_name)) +
  geom_point(size = 2.5) + geom_hline(yintercept = c(-0.7, - 3.1),
                                     linetype="dashed") +
  annotate("text", x=-32, y=-4.4, label= "Ruminant\nDairy", size=3, angle=90) +
  annotate("text", x=-32, y=-2.2, label= "Ruminant\nAdipose", size=3, angle=90) +
  annotate("text", x=-32, y=1.7, label= "Non-Ruminant", size=3, angle=90) +
  annotate("text", x=-30, y=4.2, label= "C3-input", size=3, angle=0) +
  annotate("text", x=-25, y=4.2, label= "Increasing C4-input", size=3, angle=0) +
  geom_segment(x = -27, y = 3.8, xend = -14, yend = 3.8, size = 0.5,
              arrow = arrow(length = unit(0.3, "cm")), colour = "black") +
  expand_limits(x = c(-14, -32), y = c(-6.2, 4)) +
  scale_y_continuous(breaks=c(-6, -4, -2, 0, 2, 4)) +
  scale_x_continuous(breaks=c(-34, -30, -26, -22, -18, -14)) +
  theme(plot.title = element_text(hjust=0, size=16)) +
  labs(x = expression(delta^13* C [C16:0]* " " ("\"u2030\"")),
       y = expression(Delta^13* C* " " ("\"u2030\"")),
       colour="Site name", shape = "Site name") + ggtitle("Figure 6a") +
  theme_classic() +
  scale_colour_manual(name = "Site name",
                     values = c("#440154FF", "#2D708EFF",
                                "#29AF7FFF", "#73D840FF", "#ff41ca")) +
  scale_shape_manual(name = "Site name",
                    values = c(17, 17, 17, 17, 17)) +
```

```

geom_segment(x = -30.8, y = 2, xend = -30.8, yend = 0.5, colour = "black") +
geom_segment(x = -30.8, y = -1, xend = -30.8, yend = -2.7, colour = "black") +
geom_segment(x = -30.8, y = -3.5, xend = -30.8, yend = -6, colour = "black") +
geom_segment(x = -30.6, y = 2, xend = -31, yend = 2, colour = "black") +
geom_segment(x = -30.6, y = 0.5, xend = -31, yend = 0.5, colour = "black") +
geom_segment(x = -30.6, y = -1, xend = -31, yend = -1, colour = "black") +
geom_segment(x = -30.6, y = -2.7, xend = -31, yend = -2.7, colour = "black")+
geom_segment(x = -30.6, y = -3.5, xend = -31, yend = -3.5, colour = "black") +
geom_segment(x = -30.6, y = -6, xend = -31, yend = -6, colour = "black") +
annotate ("point", x = -30.8, y = 1, colour = "black") +
annotate ("point", x = -30.8, y = -2, colour = "black") +
annotate ("point", x = -30.8, y = -5, colour = "black")
fig6a

```

## Warning: Removed 27 rows containing missing values (geom\_point).



#Figure 6b

```

fig6b <- ggplot(data_urban, aes(x = delta13C_C16, y = bigdelta,
                                colour = Site_name, shape = Site_name)) +
  geom_point(size = 2.5) + geom_hline(yintercept = c(-0.7, - 3.1),
                                     linetype="dashed") +
  annotate("text", x=-32, y=-4.4, label= "Ruminant\nDairy", size=3, angle=90) +
  annotate("text", x=-32, y=-2.2, label= "Ruminant\nAdipose", size=3, angle=90) +
  annotate("text", x=-32, y=1.7, label= "Non-Ruminant", size=3, angle=90) +

```

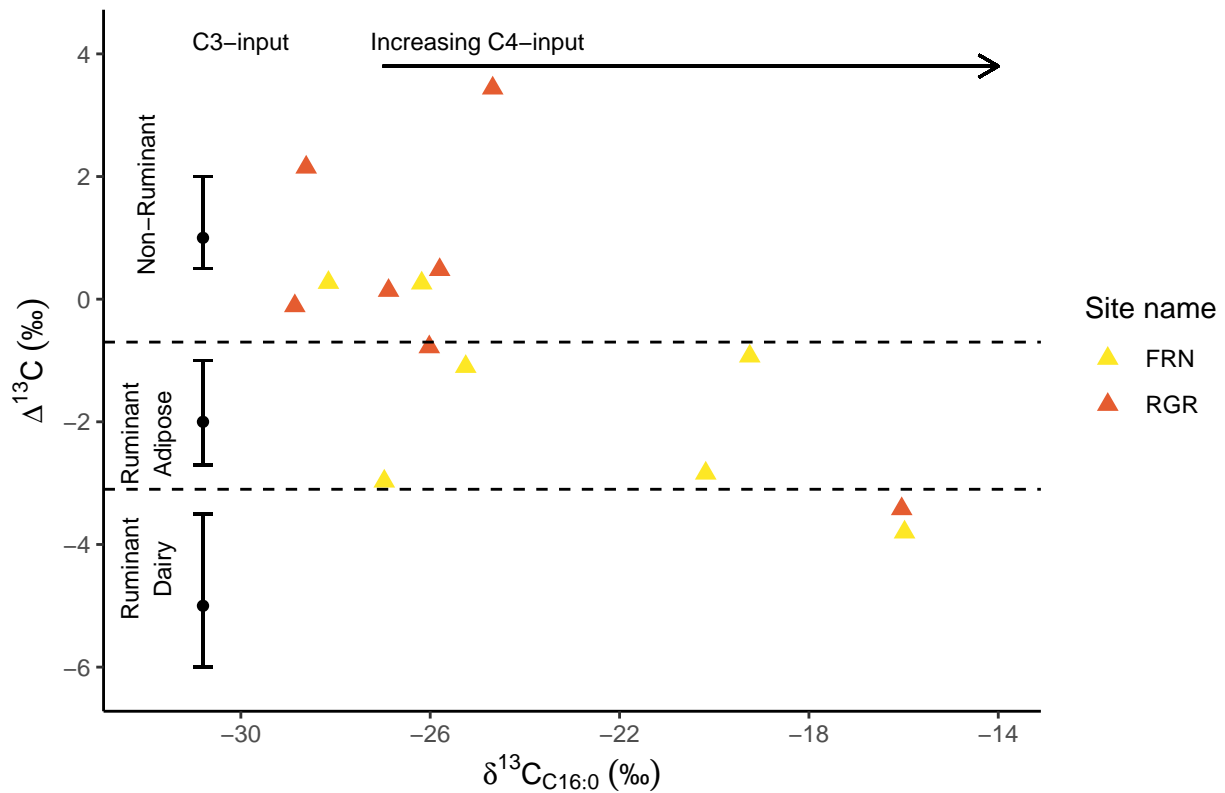
```

annotate("text", x=-30, y=4.2, label= "C3-input", size=3, angle=0)+
annotate("text", x=-25, y=4.2, label= "Increasing C4-input", size=3, angle=0)+
geom_segment(x = -27, y = 3.8, xend = -14, yend = 3.8, size = 0.5,
             arrow = arrow(length = unit(0.3, "cm")), colour = "black") +
expand_limits(x = c(-14,-32), y = c(-6.2, 4)) +
scale_y_continuous(breaks=c(-6, -4, -2, 0, 2, 4)) +
scale_x_continuous(breaks=c(-34, -30, -26, -22, -18, -14)) +
theme(plot.title = element_text(hjust=0, size=16)) +
labs(x = expression(delta13* C [C16:0]* " "("\u2030")),
     y = expression(Delta13* C* " "("\u2030")),
     colour="Site name", shape = "Site name") + ggtitle("Figure 6b") +
theme_classic() +
scale_colour_manual(name = "Site name",
                    values = c("#FDE725FF", "#e55c30"))+
scale_shape_manual(name = "Site name",
                    values = c(17, 17)) +
geom_segment(x = -30.8, y = 2, xend = -30.8, yend = 0.5, colour = "black") +
geom_segment(x = -30.8, y = -1, xend = -30.8, yend = -2.7, colour = "black") +
geom_segment(x = -30.8, y = -3.5, xend = -30.8, yend = -6, colour = "black") +
geom_segment(x = -30.6, y = 2, xend = -31, yend = 2, colour = "black") +
geom_segment(x = -30.6, y = 0.5, xend = -31, yend = 0.5, colour = "black") +
geom_segment(x = -30.6, y = -1, xend = -31, yend = -1, colour = "black") +
geom_segment(x = -30.6, y = -2.7, xend = -31, yend = -2.7, colour = "black")+
geom_segment(x = -30.6, y = -3.5, xend = -31, yend = -3.5, colour = "black") +
geom_segment(x = -30.6, y = -6, xend = -31, yend = -6, colour = "black") +
annotate ("point", x = -30.8, y = 1, colour = "black") +
annotate ("point", x = -30.8, y = -2, colour = "black") +
annotate ("point", x = -30.8, y = -5, colour = "black")
fig6b

```

```
## Warning: Removed 22 rows containing missing values (geom_point).
```

Figure 6b



#Prepare data for Figure 7

```
#change column names
names(isotope_indus)[names(isotope_indus) == "delta13C_C16"] <- "C16"
names(isotope_indus)[names(isotope_indus) == "delta13C_C18"] <- "C18"

#reshape data for boxplots
isotope_reshape<- gather(data = isotope_indus, key = d13C, value = d13C_value, C16:C18)
droplevels(isotope_reshape)
```

```
## # A tibble: 146 x 89
##   Site_name Sample Rural_urban Trench_Context Context_Notes Chronology
##   <fct>      <chr> <fct>      <chr>          <chr>          <chr>
## 1 ALM       ALM11~ Rural      SC-114         114            Late_Hara~
## 2 ALM       ALM11~ Rural      SC-117         117            Late_Hara~
## 3 ALM       ALM11~ Rural      SC-117         117            Late_Hara~
## 4 ALM       ALM11~ Rural      SC-119         119            Late_Hara~
## 5 ALM       ALM12~ Rural      SC-122         122            Late_Hara~
## 6 ALM       ALM12~ Rural      SC-124         124            Late_Hara~
## 7 ALM       ALM12~ Rural      SC-125         125            Late_Hara~
## 8 ALM       ALM12~ Rural      SC-125         125            Late_Hara~
## 9 ALM       ALM12~ Rural      SC-125         125            Late_Hara~
## 10 ALM      ALM12~ Rural      SC-125         125            Late_Hara~
## # ... with 136 more rows, and 83 more variables: Before_After_4.2_kya <chr>,
## #   Chronology_details <chr>, Artefact_type <chr>, Rim_base_body <chr>,
## #   Rim_diam_cm <dbl>, Vesseltype <chr>, Vessel_form <chr>, Vessel_type <fct>,
```



```
## # Vessel_category <chr>, Haryana_or_Classic <chr>, Saturated <chr>,
## # Unsaturated <chr>, Branched <chr>, Date_of_analysis <chr>,
## # Sample_mass_mg <dbl>, Area_GC_total <dbl>, AreaC12 <dbl>, AreaC14 <dbl>,
## # AreaC15 <dbl>, AreaC161 <dbl>, AreaC16 <dbl>, AreaC17 <dbl>,
## # AreaC181 <dbl>, AreaC18 <dbl>, AreaC19 <dbl>, AreaC201 <dbl>,
## # AreaC20 <dbl>, AreaC21 <dbl>, AreaC221 <dbl>, AreaC22 <dbl>, AreaC23 <dbl>,
## # AreaC24 <dbl>, AreaC25 <dbl>, AreaC26 <dbl>, AreaC28 <dbl>,
## # Areaphthalates <dbl>, FAMEarea <dbl>, Area_IS_C36 <dbl>, Area_IS_C34 <dbl>,
## # Mass_IS_C34_ug <dbl>, Mass_IS_C36_ug <dbl>, Lipidconc_ug_vial <dbl>,
## # Lipidconc_ug_g <dbl>, Interpretable <lgl>, C12concentration_ug_vial <chr>,
## # C14concentration_ug_vial <chr>, C15concentration_ug_vial <chr>,
## # C161concentration_ug_vial <chr>, C16concentration_ug_vial <dbl>,
## # C17concentration_ug_vial <chr>, C181concentration_ug_vial <chr>,
## # C18concentration_ug_vial <dbl>, C19concentration_ug_vial <chr>,
## # C201concentration <chr>, C20concentration_ug_vial <chr>,
## # C21concentration_ug_vial <chr>, C221concentration <chr>, 'C22concentration
## # (ug/vial)' <chr>, C23concentration_ug_vial <chr>,
## # C24concentration_ug_vial <chr>, C25concentration_ugvial <chr>,
## # C26concentration_ug_vial <chr>, C28concentration_ug_vial <chr>,
## # C34concentration_ug_vial <chr>, FAMEconcentration <dbl>,
## # Cholesterol_derivatives <chr>, 'longchain_Alkanes' <chr>, Alcohols <chr>,
## # Sulphur <chr>, qty_C16_inj_dil_100ul_ng <dbl>,
## # qty_C18_inj_dil_100ul_ng <dbl>, C16conc_ug_vial <dbl>,
## # C18conc_ug_vial <dbl>, dilution_ul <dbl>, qty_C16_inj_ng <chr>,
## # qty_C18_inj_ng <chr>, PS_ratio <dbl>, C12_C14 <dbl>, C161_C181 <chr>,
## # C15_C17_C18 <chr>, bigdelta <dbl>, d13C <chr>, d13C_value <dbl>
```

```
#reorder names of sites
isotope_reshape$Site_name <- factor(isotope_reshape$Site_name,
                                   levels = c("ALM", "MSDVII", "MSDI", "LHRI", "KNK",
                                               "FRN", "RGR"))
```

```
#Prepare data for Figure 7
```

```
#Remove Khanak because it has small sample numbers
isotope_sites <- droplevels(subset(isotope_reshape, (Site_name=="RGR") |
                                  (Site_name=="MSDI") | (Site_name=="LHRI") |
                                  (Site_name=="MSDVII") | (Site_name=="ALM") |
                                  (Site_name=="FRN")))

#set order of chronological periods
isotope_sites$Chronology_details <- factor(isotope_sites$Chronology_details,
                                           levels=c("MHi-ii", "MHiii", "LH"))

#set order of sites
isotope_sites$Site_name <- factor(isotope_sites$Site_name,
                                  levels = c("ALM", "MSDVII", "MSDI", "LHRI", "FRN", "RGR"))
```

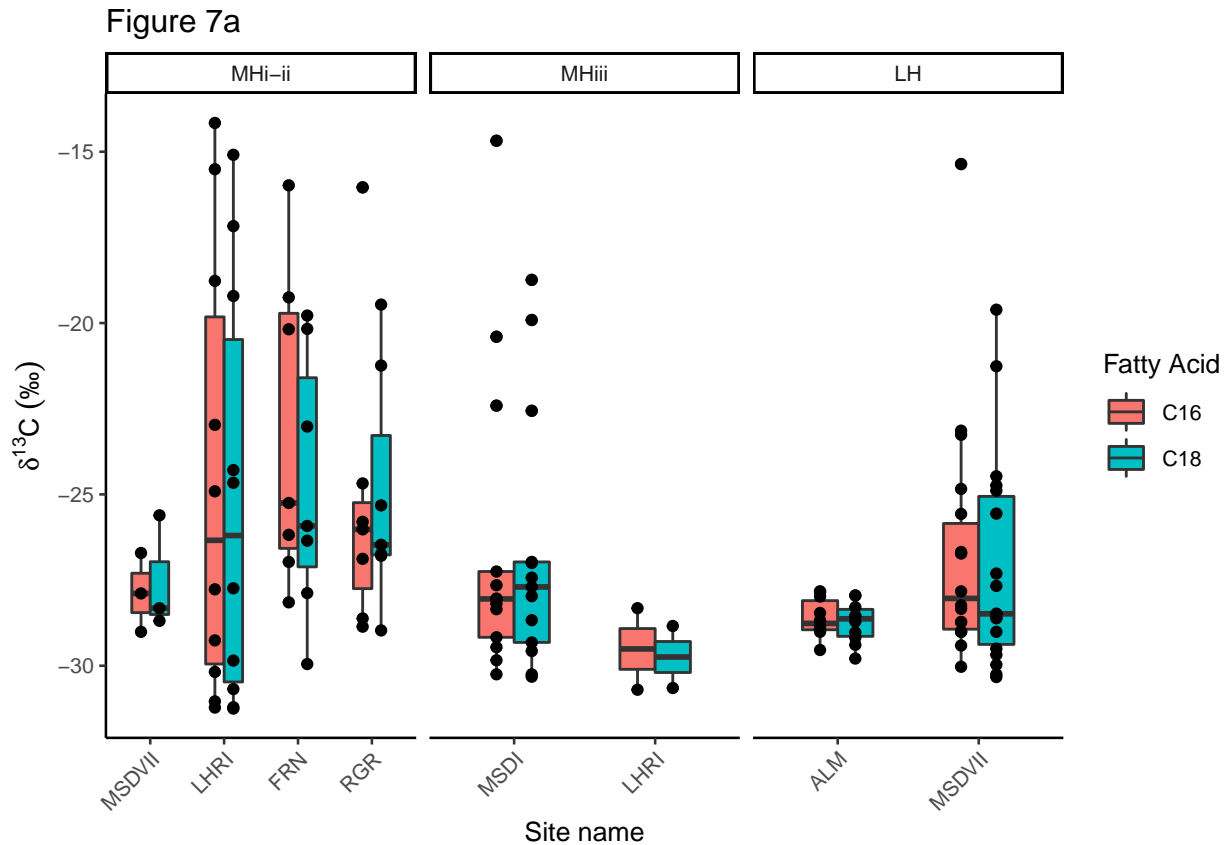
```
#Figure 7a
```

```
#Figure 7a
fig7a <- ggplot(isotope_sites, aes(x = Site_name, y = d13C_value)) +
  theme(plot.title = element_text(hjust=0, size=16)) +
```

```

labs(x = "Site name",
     y = expression(delta^13* C* " "("\u2030")), fill = "Fatty Acid") +
ggtitle("Figure 7a") +
geom_boxplot(aes(fill = d13C), position = position_dodge(0.5), width = .5) +
geom_point(aes(y=d13C_value, group=d13C), position = position_dodge(width=0.5)) +
theme_classic(base_size = 10) +
facet_wrap(~Chronology_details, scales="free_x") +
theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1))
fig7a

```



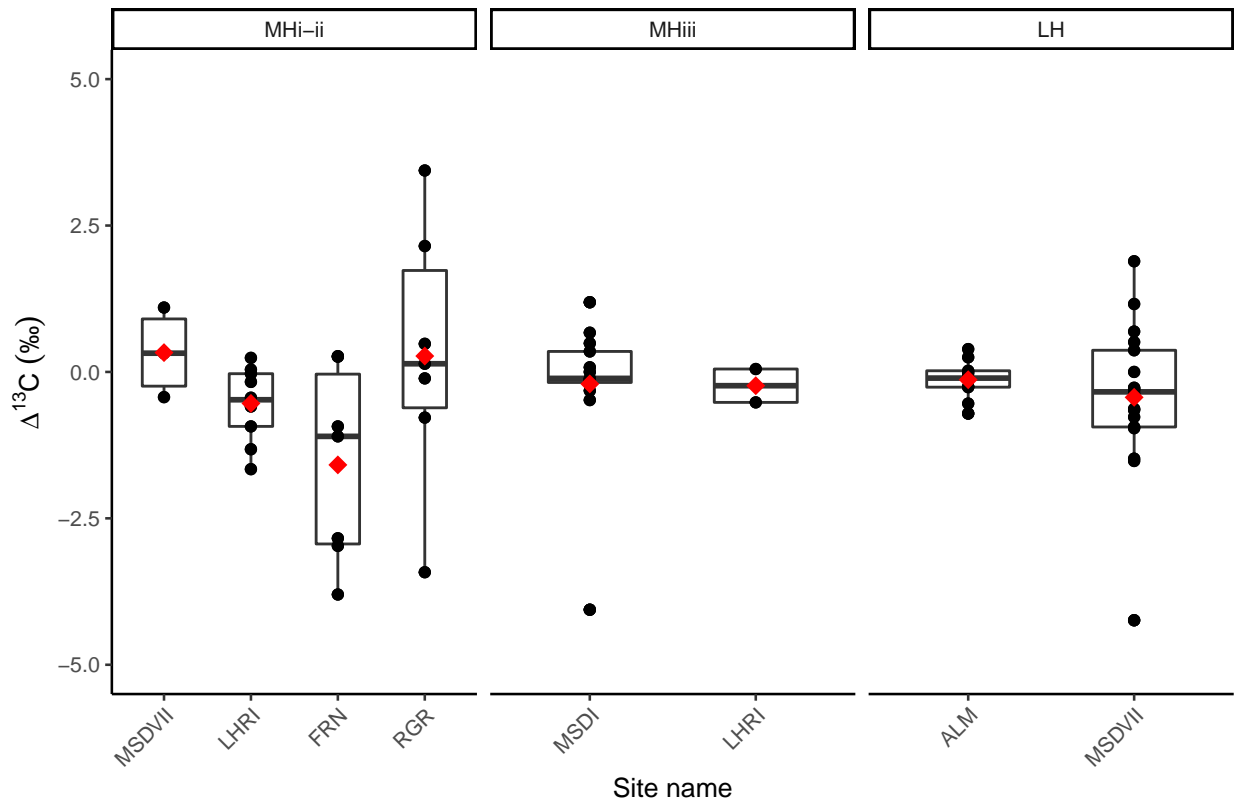
#Figure 7b

```

fig7b <- ggplot(isotope_sites, aes(x = Site_name, y = bigdelta))+
  theme(plot.title = element_text(hjust=0, size=16)) +
  labs(x = "Site name",
       y = expression(Delta^13* C* " "("\u2030"))) +
  ggtitle("Figure 7b") +
  expand_limits(y = c(-5,5)) +
  geom_boxplot(aes(), position = position_dodge(0.5), width = .5) +
  geom_jitter(aes(y=bigdelta), position = position_dodge(width=0.5)) +
  stat_summary(fun =mean, geom="point", shape=18, size=3, color="red")+
  theme_classic(base_size = 10) +
  facet_wrap(~Chronology_details, scales="free_x") +
  theme(axis.text.x = element_text(angle = 45, vjust = 1, hjust=1))
fig7b

```

Figure 7b



#Figure 8

```
fig8 <- ggplot(isotope_indus, aes(x = C16, y = bigdelta,
                                   colour = Vesseltype, shape = Vesseltype)) +
  geom_point(size = 2.5) + geom_hline(yintercept = c(-0.7, - 3.3),
                                       linetype="dashed") +
  annotate("text", x=-32, y=-4.4, label= "Ruminant\nDairy", size=3, angle=90) +
  annotate("text", x=-32, y=-2.2, label= "Ruminant\nAdipose", size=3, angle=90) +
  annotate("text", x=-32, y=1.7, label= "Non-Ruminant", size=3, angle=90) +
  annotate("text", x=-30, y=4.2, label= "C3-input", size=3, angle=0) +
  annotate("text", x=-25, y=4.2, label= "Increasing C4-input", size=3, angle=0) +
  geom_segment(x = -27, y = 3.8, xend = -14, yend = 3.8, size = 0.5,
              arrow = arrow(length = unit(0.3, "cm")), colour = "black") +
  expand_limits(x = c(-14, -32), y = c(-6.2, 4)) +
  scale_y_continuous(breaks=c(-6, -4, -2, 0, 2, 4)) +
  scale_x_continuous(breaks=c(-34, -30, -26, -22, -18, -14)) +
  theme(plot.title = element_text(hjust=0, size=16)) +
  labs(x = expression(delta^13* C [C16:0]* " " ("\"u2030")),
       y = expression(Delta^13* C * " " ("\"u2030")),
       shape="Vessel type") +
  ggtitle("Figure 8") +
  theme_classic() +
  scale_shape_manual(name = "Vessel form",
                    values = c(8, 3,
                               4, 14, 15)) +
  scale_colour_manual(name = "Vessel form",
```

```

values = c("black", "black",
           "black", "black", "red"))+
geom_segment(x = -30.8, y = 2, xend = -30.8, yend = 0.5, colour = "black") +
geom_segment(x = -30.8, y = -1, xend = -30.8, yend = -2.7, colour = "black") +
geom_segment(x = -30.8, y = -3.5, xend = -30.8, yend = -6, colour = "black") +
geom_segment(x = -30.6, y = 2, xend = -31, yend = 2, colour = "black") +
geom_segment(x = -30.6, y = 0.5, xend = -31, yend = 0.5, colour = "black") +
geom_segment(x = -30.6, y = -1, xend = -31, yend = -1, colour = "black") +
geom_segment(x = -30.6, y = -2.7, xend = -31, yend = -2.7, colour = "black")+
geom_segment(x = -30.6, y = -3.5, xend = -31, yend = -3.5, colour = "black") +
geom_segment(x = -30.6, y = -6, xend = -31, yend = -6, colour = "black") +
annotate ("point", x = -30.8, y = 1, colour = "black") +
annotate ("point", x = -30.8, y = -2, colour = "black") +
annotate ("point", x = -30.8, y = -5, colour = "black")

```

fig8

