Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes

**R Codes:**

```
##### install packages
install.packages(c("metafor","lmodel2","smatr","glm2","ggplot2"))
library(metafor,lmodel2,smatr,glm2,ggplot2)

### read data from Supplementary Dataset 1
mydata<-read.csv(file.choose())

## subset data
natural<-subset(mydata, mydata$System=="01Natural")
controlled<-subset(mydata, mydata$System=="02Controlled");controlled$biome<-"02controlled"

natNP<-subset(natural, natural$cnp=="[NP]";conNP<-subset(controlled, controlled$cnp=="[NP]"

####Figure 2
###Fig.2a: Regression meta-analysis C, N, P, N/P, C/N, C/P in natural systems
### calculate log transformed response ratio and corresponding sampling variances
natNPdat <- escalc(m1i=Xe, sd1i=Se, n1i=Ne, m2i=Xc, sd2i=Sc, n2i=Nc, measure="ROM", data=natNP, append=TRUE)
####or
yi <- with(natNP, log(Xe/Xc)); vi <- with(natNP, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
rNP<-rma.uni(yi,vi,mods=~factor(treatment)-1, method="REML", data=natNPdat, digits=8); summary(rNP)

conNPdat <- escalc(m1i=Xe, sd1i=Se, n1i=Ne, m2i=Xc, sd2i=Sc, n2i=Nc, measure="ROM", data=conNP, append=TRUE)
####or
yi <- with(conNP, log(Xe/Xc)); vi <- with(conNP, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
rNPcon<-rma.uni(yi,vi,mods=~factor(treatment)-1, method="REML", data=conNPdat, digits=8); summary(rNPcon)

####Fig.2b: interaction
naturalinter<-natural; naturalinter$sources<-paste(natural$Author,natural$Journal,sep = ")
natinterdat <- escalc(m1i=Xe, sd1i=Se, n1i=Ne, m2i=Xc, sd2i=Sc, n2i=Nc,measure="ROM", data=naturalinter, append=TRUE)
interNP<-subset(natinterdat, natinterdat$cnp=="[NP]"

NPco2<-subset(interNP, interNP$treatment=="01CO2")
NPt<-subset(interNP, interNP$treatment=="02T")
```
NP$r$ <- subset(interNP, interNP$treatment == "03R")
NP$n$ <- subset(interNP, interNP$treatment == "05N")
NP$p$ <- subset(interNP, interNP$treatment == "06P")
NP$ct$ <- subset(interNP, interNP$treatment == "07CT")
NP$cn$ <- subset(interNP, interNP$treatment == "10CN")
NP$rn$ <- subset(interNP, interNP$treatment == "17RN")
NP$np$ <- subset(interNP, interNP$treatment == "21NP")
interNP <- rbind(NP$co2, NP$t, NP$r, NP$n, NP$p, NP$ct, NP$cn, NP$rn, NP$np)

NP$control$ <- rbind(NP$co2, NP$t, NP$r, NP$n, NP$p)
NP$control$<-'CO2' <- "N"; NP$control$<-'T' <- "N"; NP$control$<-'R' <- "N"; NP$control$<-'D' <- "N";
NP$control$<-'N' <- "N"; NP$control$<-'P' <- "N"
NP$control$treatment <- "control"; NP$control$treat <- "NNNNNNN"
NP$add$ <- rbind(NP$control, interNP)

yi <- with(NP$add, log(Xe/Xc)); vi <- with(NP$add, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
NP$additive$ <- rma.uni(yi, vi, mods = ~CO2*T+R+N+P+CO2*T+CO2*N+R*N+N*P-1,
method = "REML", data = NP$add, digits = 8); summary(NP$additive)
#CO2+warming
IDinterNP$ct$ <- unique(NP$ct$sources); NP$ct$add <- interNP[1,]
for(i in 1:length(IDinterNP$ct)) {
  dat.temp <- interNP[which(interNP$sources == IDinterNP$ct[i]),]
  if (nrow(dat.temp) == 0) {next}
  else if (any(dat.temp$treatment == "01CO2") & any(dat.temp$treatment == "02T") &
  any(dat.temp$treatment == "07CT"))
    {NP$ct$add <- rbind(NP$ct$add, dat.temp)}
}

NP$ct$add <- NP$ct$add[-1,]; NP$ct$add <- NP$ct$add[order(NP$ct$add$Author),]
NP$ct$01CO2sub <- subset(NP$ct$add, NP$ct$add$treatment == "01CO2")
NP$ct$02Tsub <- subset(NP$ct$add, NP$ct$add$treatment == "02T")
NP$ct$07CTsub <- subset(NP$ct$add, NP$ct$add$treatment == "07CT")
NP$ct$add1 <- rbind(NP$ct$01CO2sub, NP$ct$02Tsub, NP$ct$07CTsub)
NP$control$ <- NP$ct$07CTsub
NP$ct$control$<-'CO2' <- "N"; NP$ct$control$<-'T' <- "N"; NP$ct$control$<-'R' <- "N"; NP$ct$control$<-'D' <- "N";
NP$ct$control$<-'N' <- "N"; NP$ct$control$<-'P' <- "N"
NP$ct$control$treatment <- "control"; NP$ct$control$treat <- "NNNNNNN"
NP$ct$add2 <- rbind(NP$ct$control, NP$ct$add1)
NP$ct$interdat <- escalc(m1i = Xe, sd1i = Se, n1i = Ne, m2i = Xc, sd2i = Sc, n2i = Nc, measure = "ROM",
data = NP$ct$add2, append = TRUE)
summary(rma.uni(yi, vi, mods = ~CO2*T, method = "REML", data = NP$ct$interdat, digits = 8));
summary(aovNP$ct$add)

#CO2+N
IDinterNP$cn$ <- unique(NP$cn$sources)
NPcnadd<-interNP[1,]

for(i in 1:length(IDinterNPcn)){
  dat.temp<-interNP[which(interNP$sources==IDinterNPcn[i]),]
  if (nrow(dat.temp)==0){next}
  else if (any(dat.temp$treatment=="01CO2") & any(dat.temp$treatment=="05N") & any(dat.temp$treatment=="10CN")){NPcnadd<-rbind(NPcnadd,dat.temp)}
}

NPcnadd<-NPcnadd[-(1),]
NPcnadd<-NPcnadd[order(NPcnadd$Author),]

NPcn01CO2sub<-subset(NPcnadd, NPcnadd$treatment=="01CO2")
NPcn05Nsub<-subset(NPcnadd, NPcnadd$treatment=="05N")
NPcn10CNsub<-subset(NPcnadd, NPcnadd$treatment=="10CN")
NPcnadd1<-rbind(NPcn01CO2sub, NPcn05Nsub,NPcn10CNsub)

NPcncontrol<-NPcn10CNsub;NPcncontrol$CO2<="N";NPcncontrol$T<="N";NPcncontrol$R<="N";NPcncontrol$D<="N";NPcncontrol$N<="N";NPcncontrol$P<="N"
NPcncontrol$treatment<="control";NPcncontrol$treat<="NNNNNN"
NPcnadd2<-rbind(NPcncontrol,NPcnadd1)
NPcninterdat<- escalc(m1i=Xe, sd1i=Se, n1i=Ne, m2i=Xc, sd2i=Sc, n2i=Nc, measure="ROM", data=NPcnadd2, append=TRUE)
summary(rma.uni(yi,vi,mods=~CO2*N, method="REML", data=NPcninterdat, digits=8) )

#Rain+N
IDinterNPrn<-unique(NPn$r sources)
NPnadd<-interNP[1,]

for(i in 1:length(IDinterNPn)){
  dat.temp<-interNP[which(interNP$sources==IDinterNPn[i]),]
  if (nrow(dat.temp)==0){next}
  else if (any(dat.temp$treatment=="03R") & any(dat.temp$treatment=="05N") & any(dat.temp$treatment=="17RN")){NPnadd<-rbind(NPnadd,dat.temp)}
}

NPnadd<-NPnadd[-(1),]
NPnadd<-NPnadd[order(NPnadd$Author),]
NPn03Rsub<-subset(NPnadd, NPnadd$treatment=="03R")
NPn05Nsub<-subset(NPnadd, NPnadd$treatment=="05N")
NPn17RNsub<-subset(NPnadd, NPnadd$treatment=="17RN")
NPnadd1<-rbind(NPn03Rsub, NPn05Nsub,NPn17RNsub)
NPncontrol<-NPn17RNsub;NPncontrol$CO2<="N";NPncontrol$T<="N";NPncontrol$R<="N";NPncontrol$D<="N";NPncontrol$N<="N";NPncontrol$P<="N"
NPrncontrol$treatment<"control"; NPrncontrol$treat<"NNNNNN"
NPrnadd2<rbind(NPrncontrol,NPrnadd1)
NPrninterdat<- escalc(m1i=Xe, sd1i=Se, n1i=Ne, m2i=Xc, sd2i=Sc, n2i=Nc, measure="ROM", 
data=NPrnadd2, append=TRUE)
someory(rma.uni(yi,vi,mods=-R*N, method="REML", data=NPrninterdat, digits=8) )

#N+P
IDinterNPnp<-unique(NPnp$sources)
NPnpadd<-interNP[1,]
for(i in 1:length(IDinterNPnp)){
  dat.temp<-interNP[which(interNP$sources==IDinterNPnp[i]),]
  if (nrow(dat.temp)==0){next}
  else if (any(dat.temp$treatment=="05N") & any(dat.temp$treatment=="06P")&
  any(dat.temp$treatment=="21NP"))
    {NPnpadd<-rbind(NPnpadd,dat.temp)}
}
NPnpadd<-NPnpadd[-(1),]
NPnpadd<-NPnpadd[order(NPnpadd$Author),]
NPnp05Nsub<-subset(NPnpadd, NPnpadd$treatment=="05N")
NPnp06Psub<-subset(NPnpadd, NPnpadd$treatment=="06P")
NPnp21NPsub<-subset(NPnpadd, NPnpadd$treatment=="21NP")
NPnpadd1<-rbind(NPnp05Nsub, NPnp06Psub,NPnp21NPsub)
NPnpcontrol<-NPnp21NPsub; NPnpcontrol$CO2<"N"; NPnpcontrol$T<-
"N";NPnpcontrol$R<"N"; NPnpcontrol$S<"N"; NPnpcontrol$N<"N"; NPnpcontrol$P<"N"
NPnpcontrol$treatment<"control"; NPnpcontrol$treat<"NNNNNN"
NPnpadd2<-rbind(NPnpcontrol,NPnpadd1)
NPnpinterdat<- escalc(m1i=Xe, sd1i=Se, n1i=Ne, m2i=Xc, sd2i=Sc, n2i=Nc, measure="ROM", 
data=NPrnadd2, append=TRUE)
someory(rma.uni(yi,vi,mods=-N*P, method="REML", data=NPrninterdat, digits=8))

####Figure 4
sCO2<-subset(natNP, natNP$treatment=="01CO2"); sT<-subset(natNP, 
natNP$treatment=="02T")
sR<-subset(natNP, natNP$treatment=="03R"); sD<-subset(natNP, natNP$treatment=="04D")
sRain<-rbind(sR,sD)
sRain$dwater<-sRain$drain+sRain$ddrought
sN<-subset(natNP, natNP$treatment=="05N"); sP<-subset(natNP, natNP$treatment=="06P")

dco2yiNP <- with(sCO2, log(Xe/Xc)); dco2viNP <- with(sCO2, (1/Ne)*(Se/Xe)^2 +
(1/Nc)*(Sc/Xc)^2)
dco2rNP<-rma.uni(dco2yiNP,dco2viNP,mods=-dCO2, method="REML", data=sCO2,
digits=8); summary(dco2rNP)
someory(aov(dco2yiNP~dCO2, data=sCO2))

dTyiNP <- with(sT, log(Xe/Xc)); dTviNP <- with(sT, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
dTrNP<-rma.uni(dTyiNP,dTviNP,mods=~dtemp, method="REML", data=sT, digits=8);
summary(dTrNP)
summary(aov(dTyiNP~dtemp, data=sT))

drainyiNP <- with(sRain, log(Xe/Xc)); drainviNP <- with(sRain, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
drainrNP<-rma.uni(drainyiNP,drainviNP,mods=~dwater, method="REML", data=sRain, digits=8); summary(drainrNP)
summary(aov(drainyiNP~dwater, data=sRain))

dNyiNP <- with(sN, log(Xe/Xc)); dNviNP <- with(sN, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
dNrNP<-rma.uni(dNyiNP,dNviNP,mods=~dN, method="REML", data=sN, digits=8); summary(dNrNP)
summary(aov(dNyiNP~dN, data=sN))

dPyiNP <- with(sP, log(Xe/Xc)); dPviNP <- with(sP, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
dPrNP<-rma.uni(dPyiNP,dPviNP,mods=~dP, method="REML", data=sP, digits=8); summary(dPrNP)
summary(aov(dPyiNP~dP, data=sP))

sCO2con<-subset(conNP, conNP$treatment=="01CO2"); sTcon<-subset(conNP, conNP$treatment=="02T")

dco2yiNPcon <- with(sCO2con, log(Xe/Xc)); dco2viNPcon <- with(sCO2con, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
dco2rNPcon<-rma.uni(dco2yiNPcon,dco2viNPcon,mods=~dCO2, method="REML", data=sCO2con, digits=8); summary(dco2rNPcon)
summary(aov(dco2yiNPcon~dCO2, data=sCO2con))

dTyiNPcon <- with(sTcon, log(Xe/Xc)); dTviNPcon <- with(sTcon, (1/Ne)*(Se/Xe)^2 + (1/Nc)*(Sc/Xc)^2)
dTrNPcon<-rma.uni(dTyiNPcon,dTviNPcon,mods=~dtemp, method="REML", data=sTcon, digits=8); summary(dTrNPcon)
summary(aov(dTyiNPcon~dtemp, data=sTcon))

############################# The end
Table S1
Sensitivity of plant stoichiometry to global change treatments in different ecosystems. The mean log response ratio (lnRR) with the 95% confidence intervals and number of observations in parentheses are reported for biome with the number of observations ≥ 15. CO2, Warming, Rainfall+, Rainfall-, Nitrogen+, and Phosphorus+ refer to field experiments of elevated [CO2], increasing temperature, increasing rainfall, decreasing rainfall, nitrogen fertilization, and phosphorus fertilization, respectively. 0 represents the value <0.01. lnRR significantly different from zero (P<0.05) are shown in bold.

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<th>CO2</th>
<th>Warming</th>
<th>Rainfall+</th>
<th>Rainfall-</th>
<th>Nitrogen+</th>
<th>Phosphorus+</th>
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<tr>
<td>Temperate forest</td>
<td>0.028±0.009</td>
<td>-0.016±0.01</td>
<td>0±0</td>
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<tr>
<td></td>
<td>(431)</td>
<td>(87)</td>
<td>(55)</td>
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<tr>
<td>Tropical forest</td>
<td>0.224±0.033</td>
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<tr>
<td>Temperate</td>
<td>0.003±0.01</td>
<td>0.045±0.039</td>
<td>0±0.041</td>
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<tr>
<td>grassland</td>
<td>(221)</td>
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<td>(26)</td>
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<tr>
<td>Tundra</td>
<td>0.037±0.017</td>
<td>0.047±0.023</td>
<td>0.053±0.004</td>
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<tr>
<td>Wetland</td>
<td>0.047±0.02</td>
<td></td>
<td>-0.163±0.098</td>
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<td>Crop</td>
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<td>-0.178±0.045</td>
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<tr>
<td>Desert</td>
<td>-0.045±0.037</td>
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<tr>
<td>N:P</td>
<td>-0.062±0.05</td>
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<tr>
<td>Temperate</td>
<td>0.496±0.103</td>
<td>0.013±0.083</td>
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<tr>
<td>grassland</td>
<td>(54)</td>
<td>(44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tropical grassland</td>
<td></td>
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<tr>
<td>Tundra</td>
<td>-0.004±0.005</td>
<td>-0.068±0.029</td>
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<tr>
<td>Wetland</td>
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Figure S1
The global distribution of study sites included in the meta-analysis.
Figure S2
Natural log response ratios of plant nutrients and their stoichiometric ratios to six global change treatments. a, Log response ratios of plant concentrations of carbon ([C]), nitrogen ([N]), and phosphorus ([P]). b, Log response ratios of C:N, C:P, and N:P. Circles are for results in natural environments with grey and green representing insignificant ($P > 0.05$) and significant ($P \leq 0.05$) difference between the log response ratio and zero, respectively. Triangles are for results in controlled environments with grey and pink representing insignificant ($P > 0.05$) and significant ($P \leq 0.05$) difference between the log response ratio and zero, respectively. Error bars are the 95% confidence intervals for the mean. CO2, Warming, Rainfall+, Rainfall-, Nitrogen+, and Phosphorus+ represent elevated [CO2], increasing temperature, increasing rainfall, decreasing rainfall, nitrogen fertilization, and phosphorus fertilization, respectively. Overall response ratios were calculated by pooling all data for each of the six studied plant nutrient variables. The numbers out- and inside parentheses represent the numbers of observations for experiments in natural and controlled environments, respectively.
Figure S3

Natural log response ratios of plant nutrients and their stoichiometric ratios to combined global change treatments. The uses of symbols, colours, error bars, and abbreviations are same as in Fig. 2. Only combined experiments with $n \geq 15$ are included. Abbreviations are same as in Supplementary Fig. S2.
Figure S4
Parameter estimates (mean and the 95% confidence intervals) for two-way interaction terms between global change treatments. The parameters are estimated by fitting generalized linear mixed-effect models. Insignificant interaction terms indicate that treatment effects are additive. Only studies reporting single and two-factor experiments are included in these analyses. Abbreviations are same as in Supplementary Fig. S2.
**Figure S5**

*Sensitivities of natural log response ratios to quantities of global change treatments.* The uses of symbols, colours, error bars, and abbreviations are same as in Fig. 2. Because application rates of water, nitrogen and phosphorus fertilization can not be standardized to same units in controlled environments, only the responses to elevated [CO₂] and warming treatments in controlled environments are presented. Abbreviations are same as in Supplementary Fig. S2.
Figure S6
Relationships between N:P response ratios and aridity. Aridity index, 1-the ratio of precipitation to potential evapotranspiration, for each site of manipulation experiments is derived from the CGIAR-CSI (http://www.cgiar-csi.org/data/global-aridity-and-pet-database). The fitted regression line for Rainfall+ is significantly quadratic ($y = -0.12 + 0.21x - 0.087x^2$, $n = 116$, $R^2 =0.31$, $P =0.003$). The fitted regression line for Rainfall- is not significant ($y = -0.052 + 0.11x$, $n =26$, $R^2 =0.28$, $P =0.15$). 

![Graph showing the relationships between N:P response ratios and aridity](image-url)
Figure S7
Response ratios of plant [C] among plant functional types. The symbol with error bar shows the mean response ratio with the 95% confidence intervals. Dec. ang. and Evergr. ang. refer to deciduous woody angiosperms and evergreen woody angiosperms, respectively. Abbreviations are same as in Supplementary Fig. S2.
Figure S8
Response ratios of plant [N] among plant functional types. Abbreviations are same as in Supplementary Fig. S2.
Figure S9
Response ratios of plant [P] among plant functional types. Abbreviations are same as in Supplementary Fig. S2.
Figure S10
Response ratios of plant C:N among plant functional types. Abbreviations are same as in Supplementary Fig. S2.
**Figure S11**
Response ratios of plant C:P among plant functional types. Abbreviations are same as in Supplementary Fig. S2.
Figure S12
Response ratios of plant N:P among plant functional types. Abbreviations are same as in Supplementary Fig. S2.
Figure S13
C₃ (triangle) and C₄ (circle) plant response ratios of plant N:P to global changes.
Abbreviations are same as in Supplementary Fig. S2.
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